13th National Congress of Parasitology

FEBRUARY 24 - 26, 1999

SOUVENIR & ABSTRACTS

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13th NATIONAL CONGRESS OF PARASITOLOGY

February 24-26, 1999

Bangalore University

&

The Indian Society for Parasitology

Souvenir & Abstracts

Organised by :

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Bangalore University
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PREFACE

The Indian Society for Parasitology is organising its annual convention at Centre for Applied Genetics of Bangalore University. On behalf of the National Executive and Local Organising Committee, I take this opportunity to extend a warm welcome to all the delegates, invited guests, attend in the "13th National Congress of Parasitology" at 'Yavanika' Auditorium, Nrupathunga Road, Bangalore, from 24 to 26th February, 1999.

Bangalore, capital city of the State of Karnataka is known both as the 'Science City' as well as the 'Silicon Valley' of India. With a rich cultural heritage and massive industrial growth, it is one of the fastest growing city in the Nation.

The congress is being attended by over 300 delegates from all over the country. The scientific programmes have been arranged so as to allow maximum time to make deliberations and discussions and to spend time to observe the current progress achieved in research through the posters.

We look forward to a successful conference which will go a long way in the control of parasitic diseases.

I once again welcome you all to Bangalore and to the congress and look forward for your active participation in the deliberations of the 13th National Congress of Parasitology.

Feb, 18th, 1999

Dr. N. J. Shetty
Chairman and Organizing Secretary
13th National Congress of Parasitology

13th NATIONAL CONGRESS OF PARASITOLOGY

February 24-26, 1999

Programme

Wednesday - February 24, 1999

08.00 Hrs.	Registration
09.30 Hrs.	Inauguration
11.00 Hrs.	TEA
11.30 Hrs.	Keynote Address
12.00 Hrs.	Special Lecture
12.30 Hrs.	LUNCH
14.00 Hrs.	Scientific Session - I
	(Sponsored by ASTRA Research Centre India)
16.00 Hrs.	TEA
16.15 Hrs.	Scientific Session - II
18.00 Hrs.	At Home (Cultural Programme)
19.45 Hrs.	DINNER

Thursday - February 25,1999

09.00 Hrs.	Inauguration (Workshop & Exhibition)
10.00 Hrs.	Scientific Session - III
	Plenary / Invited Lectures
10.45 Hrs.	TEA
11.00 Hrs.	Plenary / Invited Lectures - Contiuation
13.00 Hrs.	LUNCH
	Poster Session - I
15.00 Hrs.	TEA
15.15 Hrs.	Scientific Session - IV
18.00 Hrs.	Session for Young Scientist Award
19.30 Hrs.	DINNER

Friday, February 26,1999

08.30 Hrs.	Scientific Session - V
09.45 Hrs.	Scientific Session - VI
11.00 Hrs.	TEA
11.15 Hrs.	Scientific Session - VII
13.00 Hrs.	LUNCH
	Poster Session - II
15.00 Hrs.	Valedictory Function / Concluding Session
17.00 Hrs.	HIGH TEA

THE CONGRESS IS SPONSORED BY :

- **BANGALORE UNIVERSITY**, Bangalore
- **THE INDIAN SOCIETY FOR PARASITOLOGY**, Bangalore
- **COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH**, New Delhi
- **INDIAN COUNCIL FOR MEDICAL RESEARCH**, New Delhi
- **DEPARTMENT OF SPACE, GOVERNMENT OF INDIA**, Bangalore
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- **KARNATAKA SOAPS AND DETERGENTS LTD.**, Bangalore
- **ASTRA RESEARCH CENTRE INDIA**, Bangalore
- **STERLING LAB.**, Bangalore

PLENARY / INVITED LECTURES

KEYNOTE ADDRESS

1

A NEW DRUG TARGET IN THE MALARIAL PARASITE

G.Padmanaban
Department of Biochemistry, Indian Institute of Science, Bangalore 560 012

Chloroquine resistance in the malarial parasite is rapidly spreading and the alternate drug combinations are not as effective and have side effects. The Chinese drug, artemether/artether, has to be used sparingly in cerebral malaria and resistance to this drug is also being reported. Therefore, it has become necessary to identify newer drug targets before new drugs can be designed. Studies in this laboratory have shown that the malarial parasite can synthesize heme *de novo*, despite acquiring large amounts of heme from the host hemoglobin. Inhibition of the *de novo* pathway for heme biosynthesis in the parasite leads to its death. However, the pathway operates in the host as well and therefore, a selective target is needed. Further studies reveal that the parasite has the gene only for the first enzyme, δ-aminolevulinate (ALA) synthase, but it actually imports the host ALA dehydrase and perhaps the subsequent enzymes to carry out heme biosynthesis. A receptor on the parasite membrane binding to host ALAD has been identified. It has been possible to inhibit the binding to host ALAD to the parasite membrane and also block its import. This leads to a fall in the ability of the parasite to synthesize heme leading to its death. Thus, a unique step, namely import of host ALAD into the parasite has been identified as a new drug target.

2

LEISHMANIASIS:EPIDEMIOLOGY IN INDIA AND DIAGNOSIS

R.C.Mahajan
Department of Parasitology, Postgraduate Institute of Medical Education & Research, Chandigarh.

Visceral leishmaniasis is of major public health importance in several tropical and subtropical countries including India. It is endemic in 47 countries and about 200 million people are at risk with about 5000 deaths occurring annually. In India it is mainly endemic in states of Bihar, northern part of West Bengal and eastern part of U.P, Small foci have also been reported from some other parts of the country. The number of cases have shown increase from 22739 in 1981 to 77102 in 1992 and 21884 cases with 274 deaths in 1995. Diagnosis, apart from the classical clinical features in a patient coming from an endemic area depends on the demonstration of LD bodies in splenic or bonemarrow aspirate/ lymph node aspirate or by culture. Detection of antileishmanial antibodies by IHA, IMF or ELISA using purified promastigote or amastigote antigen is useful but is of limited value in differentiation of the present from the past infections and so also in the subclinical stage or in acute stage of the disease. Test employing K39 recombinant antigen has been found to give more sensitive and specific results. DAT is good for conducting seroepidemiological surveys. Demonstration of specific antigen by using monoclonal antibody against 55kd appears to be more specific. PCR if done properly has been found to be more sensitive and specific and has been recommended as a gold standard test for diagnosis of visceral leishmaniasis.

3

MALARIA VACCINE DEVELOPMENT

V.S.Chauhan,
International Centre for Genetic Engineering & Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067

Malaria is endemic throughout most of the tropical world where more than two billion people are exposed to infection. It is estimated that approximately 500 million malaria cases occur now each year resulting in more than two million deaths, mostly in children. Widespread and increasing resistance of the parasite to antimalarial drugs, development of resistance of *Anopheles* mosquito vectors to commonly used insecticides, an inadequate infrastructure for delivery of control measures, population growth, and movement of non-immune populations to malarious areas have all contributed to the persistence and in many cases, worsening of the malaria problem. Given this situation, it is believed that vaccine should be a useful addition to chemotherapy and the vector control program in malaria control.

Malaria immunity is slow to acquire and is usually short lived. Highly polymorphic nature of malaria antigens within each parasite species and antigen structures are proposed to be the main reasons for the slow and transient nature of the acquired immunity. Further, the immunity is both species and stage specific. Notwithstanding this lack of naturally acquired sterile protective immunity, there are several reasons to believe that malaria vaccines are biologically possible. Malaria vaccines against all the three distinct developmental stages of the parasite are being developed. Vaccines against the pre-erythrocytic stages of malaria aim to eliminate infection by blocking sporozoites from entering hepatocytes or by destroying the infected hepatocytes. The second type of vaccine targeted against the blood stages of the parasite which would be expected to prevent the disease or significantly reduce the parasite load and therefore the intensity of infection. The third type is aimed at the sexual stages of parasite and aims to limit transmissions of the disease. Several antigens and different strategies have been explored but a successful malaria vaccine has not yet been developed. Recent advancements in this field and our own work at ICGEB will be presented in detail.

4

DIAGNOSIS AND IMMUNOMONITORING FOLLOWED BY OpDEC THERAPY IN MANAGEMENT OF LYMPHATIC FILARIASIS IN AN ENDEMIC AREA

B.C.Harinath, M.V.R.Reddy and *V.K.Mehta

*J B Tropical Disease Research Centre & Department of Biochemistry and *Department of Surgery, MGIMS, Sevagram 442 102 (Wardha)*

Human lymphatic filariasis caused by major nematode parasite *Wuchereria bancrofti* is a major public health problem in India with about 20 million people suffering from clinical manifestations of the disease, 7.5 million with lymphoedema and 13 million with hydrocele. Atleast twice the number suffer with occult filarial infection in endemic areas without diagnosis. The clinical cases usually do not show microfilaraemia and thus the parasitological diagnosis based on demonstration of microfilariae in the night blood sample is not useful in clinical cases. Hence, various immunodiagnostic assays have been explored based on detection of antibodies using specific filarial antigens or on detection of parasite antigen in circulation. Studies in our laboratory have shown the usefulness of filarial antibody and antigen assays using microfilarial excretory-secretory (mf ES) antigen in detecting acute and chronic filarial cases and in confirming filarial aetiology in occult infections. Diethylcarbamazine citrate (DEC) is the drug of choice for lymphatic filariasis. Different regimens of DEC have been effectively used in the treatment of microfilaraemic cases. Immunomonitoring showed that the seroconversion of antigen and antibody positively was found to be very helpful in determining appropriate period of DEC treatment. With optimal DEC therapy (OpDEC therapy), the clinical filarial patients experienced clinical relief and cure and further did not have recurrence in most of the cases. OpDEC therapy was found to be very effective in acute and atypical clinical manifestations such as asthmatic bronchitis, pulmonary eosinophilia, monoarthritis, recurrent URI, pneumonia (super imposed infections) in children and minimal hydrocele, epididymoorchitis, lymphangitis, lymphadenitis, acute abdomen, central serous retinopathy, tenosynovitis, pain and swelling in limbs and joints in adults living in filaria endemic areas.

5

SOME CRITICAL ISSUES OF IPM IN THE CHANGING AGRICULTURAL SCENARIO IN INDIA

G.K.Veeresh
No. 239, 4th Main, Ganganagar, Bangalore 560 032

The effects of green revolution of Sixties and Seventies have reflected the dark side of the success in the Eighties and Nineties when large number of farmers in India reached a crisis stage, so much so that some farmers went to the extent of committing suicide. This was not altogether unexpected to those who had the knowledge of some South American Countries farmer's plight in Sixties, who saw the sudden rise in the yields with the use of fertilizers and pesticides in cotton, soon found not only the yields declining but also the pests became uncontrollable and reached a disastrous stage. The voice raised by a few in India were drowned in the euforia of high yields and neither the farmers nor the extension mechanism were in a mood to foresee the danger.

It took less than three decades to do the damage, not only to the agro-ecological conditions of the country but the very philosophy of the eco-friendly sustainable agriculture followed by our farmers for centuries. Now it needs the patience, courage and capability of planners, scientists and farmers to reverse the trend without sacrificing the yields.

Are there any means and methods tested and verified, available to recommend to the farmers to adopt sustainable practices of INM and IPM? Yes, provided we have the will to do. INM and IPM are inseperable. These two have not been integrated properly because of which more often IPM has not given the expected results. Our training of IPM in class rooms has been an academic exercise without the background knowledge of field situations. Now the situations changing for good and lot of information has been generated and are available for application in the field. However, it requires a strong extention mechanism backed up with expertise and skill and the will of the people.

6

JAPANESE ENCEPHALITIS IN INDIA: CERTAIN ENTOMOLOGICAL ASPECTS

D.T. Mourya
National Institute of Virology, 20 A, Dr. Ambedkar Road, Pune 411 001

Japanese encephalitis (JE) is a vector-borne viral disesase. In recent years, due to its spread in newer areas it has gained considerable attention as an important emerging rural public health problem in various parts of the country. Various species of culicine mosquitoes are incriminated as vectors of this virus in South East Asia. One of the foremost intriguing questions in the epidemiology of JE is what makes a mosquito species act as a vector in an area? This question assumes complexity due to the compiex nature of fauna and highly variable climatic factors. Vector species based studies on clearly defined scientific parameters have not been undertaken in many areas in the past. Therefore, determination of the vector species is still considered as the primary objective of monitoring the mosquito species involved in virus transmission in an area.

In India, studies in the past on several mosquito species have ranked *Culex tritaeniorhynchus, Cx. pseudovishnui* and *Cx. vishnui* as important vectors. These mosquito species have aslo shown their capability of virus transmission in the laboratory. However, JE virus isolation records show that a majority of the isolates come only from *Cx. tritaeniorhynchus* mosquitoes. In many instances it has been found that though this species was predominantly present it did not play an important role in transmission. Our knowledge of understanding the *Cx. vishnui* species complex is based on morphological characters. If newer molecular approaches are used to distinguish these species it may give a better clue to understand how a species acts as a vector in a specific area. Similar studies have already been carried out in the case of sub-species complex of *Anopheles culicifacies* in our country.

Several field and laboratory studies conducted on the possibility of transovarial transmission (TOT) of this virus have added a new dimension in the natural cycle of JE. This phenomenon was first dem-

onstrated in the laboratory in certain species of culicine mosquitoes; subsequently its occurrence was confirmed in nature. Today, many studies carried out in our country have shown its true epidemiological importance in the natural cycle of JE and its possible use in monitoring JE. Advanced studies on TOT not only help in monitoring JE but also show which are the species involved in transmission in an area by knowing the minimum infection rates (MIR) in nature. The occurrence of venereal transmission of JE in mosquitoes has added another dimension to the persistence of virus in the inter-epidemic seasons.

There has been a wide variation in the clinical to sub-clinical ratios in south, north and northeastern regions of our country. The role that mosquito vectors have played in this is not understood. Besides other parameters, it appears that intrinsic factors of vector competence like threshold of infection, time period required for virus to reach the glands and extent of glands infection may affect the transmission capability of a species or a strain of the vector. Perhaps determination of MIR in different geographical strains of a vector species coupled with detailed vector competence studies may help us in understanding this fact.

Similarly, studies have been carried out to understand the effect of a climatic factor like temperature on the vectors. There has been a direct relationship of temperature and incubation period of virus in the vectors. A sudden change in temperature in an area can drastically affect the structure of vector population. The use of pesticides also affects the vector dynamics. All studies in the past have been directed on the effect of temperature and virus vector relationship in the adult mosquitoes. Very little consideration has been given to understand its possible effect on the adults emerging from the immatures, which have experienced these two stresses. It has now been clearly shown that MIR in the immature stages and adults are much different in the same area but their direct association with the climatic factors has not been shown.

These entomological factors are not only important to understand the virus-vector relationship but would also be useful in formulating a better vector monitoring system for our understanding of the natural cycle of this disease.

7

USE OF HIGH THROUGHPUT SCREENING IN THE DEVELOPMENT OF NEW ANTIMALARIALS

Santanu Datta
Astra Biochemicals India, 277, T. Chowdiah Road, Malleswaram, Bangalore 560 003

The pathway to the development of candidate drugs has undergone a sea change in the last few years. With the advent of robotics it is now possible to screen in a day several thousands of compounds for their potential efficacy. The central role in this scheme of operation is a robust enzyme assay, and screen is set up to identify potential inhibitors. The choice of the enzyme is made on the proven concept that if an essential enzyme of pathogen/disease process is inhibited, then the pathogen is eliminated or the disease process is arrested.

We have used this concept to develop a robust assay for the enzyme Hypoxanthine Guanine Phopsphoribosyl Transferase of the malarial parasite *Plasmodium falciparum* (Pf HGPRT). Pf HGPRT is an essential enzyme in the salvage pathway of the parasite which lacks the *de novo* pathway of purine synthesis. In order to set up the assay, recombinant Pf HGPRT and Human inosine monophosphate dehydrogenase (IMPDH) were cloned, expressed and purified. The assay was set up using Biomeck 2000 robot and used to screen several thousand of compounds. Potential inhibitors were identified and the inhibitors were tested for antimalarial activity.

This presentation will deal with the concepts that are used in selecting target enzymes for potential robotic screening and how the enzyme inhibitors are filtered through a process into a potential candidate drug.

8

IMMUNITY AND IMMUNOPROPHYLAXIS IN AVIAN COCCIDIOSIS

V.S. NARSAPUR

Departments of Pathology, Bacteriology, Parasitology, Food Hygiene & Public Health and Radio Isotype Labs, Bombay Veterinary College, Parel, Mumbai 400 012

Acquired immunity specific to *Eimeria* sp is known feature of coccidiosis. Among various antigens produced by coccidia, some are common to all stages, few are immunodominant initiating protection, fewer are shared antigens between different species of *Eimeria*, while very few detected so far are both shared and protective. A search for more antigens of the last category is continued.

Candidate antigens are identified on the basis of their action on T cell proliferation and induction of gamma interlukin IFN-Y. Present information indicates that most of these epitopes are located on surface of sporozoites more so at the apicomplex region

Immune responses generally occur in the gut asociated lymphoid tissue of the birds. Antigens being macrophage dependent, induce both T and B cell mediated responses.

Humoral and secretory antibodies have a minor role in protection. Material antibodies against certain purified antigens however protect chicks against challenge. In conferment of protective immunity T cells (CD8 + and TCR β+), lymphokines and cytokines(IFN-Y, MAF and TNF like factor), NK cells and macrophages have central role.

Among various vaccination procedures using virulent and attenuated strains, vaccination with polyvalent precocious strains is found superior. However, sub unit vaccines produced by recombinant techniques holds a bright hope for future. Genes encoding 13 immunodominant protein molecules have been identified and one of these is common to all major species of *Eimeria* affecting poultry. A number of vectors (high expression bacteria and viruses) have been used with success to obtain recombinant protein antigen molecules.

Other areas being explored to overcome problems of coccidiosis are, i) Use of cytokines ii) Maternal antibodies and iii) Breeding of resistant birds. Next millenium would start great advantages to poultry industries against coccidiosis.

9

THE USE OF GENETICS IN VECTOR CONTROL

N.J. Shetty
Centre for Applied Genetics, Bangalore University, Jnanabharathi Campus, Bangalore 560 056

Insecticide research led to the first, "Complete" - victories in combating pest almost 50 years ago with chlorinated hydrocarbons followed by the organophosphates, methyl carbamates, and pyrethroids - All neuro active chemicals. The very wide spread use of insecticides for pest control is largely a result of their convenience, simplicity, effectiveness, flexibility and economy. However, the insect vectors have developed resistance for the insecticide and their continuous use contributed to environmental pollution. Therefore, it is imperative that alternative control measures may be developed which does not involve resistance. Genetic control is one such method.

The vector control through genetic manipulation requires establishment of basic genetic information which could be achieved through establishment of genetic markers, cytological markers, etc., in order to conduct basic and applied research of the target species. This paper reviews the use of mutant markers, inversion polymorphism and other genetic informations which could be directly applied for the control is substantiated by taking two model mosquito vectors such as *Anopheles stephensi* Liston and *Culex* pipens *quinquefasciatus*, principal vectors of malaria and filaria respectively.

Genetic sexing strain involving male linked translocations, conditional lethals and incorporation of crossover suppressors can be utilised to produce only males and thereby it can be used in the Sterile Insect Technique (*SIT*) programme of insect vectors of diseases. Benefical genes such as genes for refractoriness can be used in the production of transgenic strain and thereby used in the control of insect vector of diseases.

10

TISSUE STAGE OF MALARIA PARASITE

K.K. Kamboj
Division of Parasitology, Central Drug Research Institute, Lucknow 226 001

Research on tissue stages of malaria parasite has been greatly hampered due to their non-accessibility being located in a deeper organ, the liver. Recently developed methods of culturing these forms outside the host have greatly augmented research on these stages. The exoerythrocytic (EE) forms have now been successfully cultured in a variety of hepatoma cell lines and primary hepatocyte cultures. Highly sensitive immunological and molecular methods have been developed to quantify the effect of various factors on these stages. The usefulness of these systems in malaria research will be discussed.

11

BIOCONTROL OF INSECTS AFFECTING LIVESTOCK

G. Karunamoorthy
University Training & Research Centre, Tirupur 641 604, T.N.

Insects of livestock are notorious as nuisance to livestock. They are responsible for skin reactions, blood loss, reduced feed conversion, poor weight gain and decreased production performance in livestock. Moreover, they transmit pathogenic organisms to the livestock either mechanically or biologically. Biocontrol by employing pathogens parasitoids and predators for keeping insect populations at a considerably low level will be discussed.

12

REMOTE SENSING OF MALARIAGENIC ENVIRONMENT

S. Adiga* and P. Manavalan**

** Director, NNRMS-RRSSC, ISRO HQ, Bangalore 560 094*

*** Scientist, RRSSC, Bangalore 560 070*

Remote sensing is gathering of information about the objects and features without placing instruments / sensors in contact with them. The remote sensing satellites which are important spaceborne platforms, offer unique advantages like repetitive and large area coverage, providing reliable, timely and comprehensive data on terrain details. Recent developments in the sensor technology, availability of improved data and analysis of remotely sensed landscape details such as vegetation, swampy areas, etc., along with other data sources within a geographical information system (GIS) have opened up new possibilities in malaria stratification, monitoring and early warning. The remote sensing approach to the study of vector borne disease is based on the identification of environmental factors that determine the temporal and spatial distribution of both vectors and diseases. Factors such as elevation, temperature, rainfall and humidity influence the presence, development, activity and longevity of malaria vectors, as well as the development of malaria parasites within vectors. Vegetation type and distribution are also determined by these variables and influence vector populations as well. Hence, vegetation, as expressed by landscape element can be used to predict the distribution and abundance of certain vector mosquitoes.

Using Indian Remote Sensing Satellite (IRS) LISS-II data, identification and monitoring of aquatic environment around Delhi, which support the thriving of mosquito population has been done. Six study sites are chosen and the data on larva and adult mosquito densities are collected at sample locations on concurrent dates of satellite overpass. Digital mapping of spatial and temporal variation of geo-environmental categories has been carried out and related with changes in mosquito population densities. The analysis of data from PAN (5.8m)

and LISS III (23 m in 3 bands) sensors of IRS-1C/1D satellite is expected to provide much improved results in this regard. In another study, the role of remote sensing and GIS in mapping the potential breeding sites of mosquitoes in Chandrapur Taluk of Maharashtra, involving spatial and non-spatial data base has been demonstrated.

The application of remote sensing techniques to malaria control has generally been focussed on the identification of mosquito habitats rather than on the clinical consequences of vector, parasite and human contact. However, in a recent study (1998) for Kenya in Africa, the characteristically seasonal fluctuations in clinical malaria, in relation to a variety of surrogate meteorological and vegetation variables recorded by sensors on board polar-orbiting and geostationary satellites has been analysed and clinical disease seasonality maps are produced. In another attempt on assessing the risk of disease transmission in the portion of Pacific coastal plain of southern Chiapas, Mexico, the use of remote sensing and spatial analysis techniques to identify and map landscape elements that collectively define vector and human population dynamics has been investigated.

Thus, the potential of remote sensing techniques for use in malaria control (for stratification, monitoring and early warning) is being tested in a number of malaria-endemic regions. With the continuous improvements in spectral and spatial (a few metres) resolutions of spaceborne sensors, much enhanced results could be obtained in these areas. Using satellite data within a GIS, provides an opportunity to integrate up-to-date environmental information, local knowledge and recent historical trends in a way that draws attention to areas of change and potential problems.

13

MALARIA CONTROL BY GENETIC MANIPULATION OF VECTORS: STATUS AND RESEARCH NEEDS IN INDIA

Sarala K. Subbarao
Malaria Research Centre, 22, Sham Nath Marg, Delhi 110 054

Target species specific control is widely accepted method today. In this context genetic control methods have a place in vector disease control programmes. In these programmes, control is achieved either by release of sterile insects to eliminate the natural population or to replace the natural population with genetically engineered or manipulated one that is incapable of transmitting malaria. The former is suitable for species with isolated distribution. However, the latter is getting more acceptance from researchers as majority of the vector species have continuous distribution in vast areas. The "replacement strategy" requires research and development in three major areas: (i) The identification of 'parasite inhibiting genes, (ii) The development of technology for introducing such genes into the genome of vector species and (iii) The development of methods to introduce engineered genomes into the natural populations.

The last few years have seen tremendous advances in the recombinant DNA technologies. It is these advances which have given hope for the prospect of developing transgenic mosquitoes. Many research groups all over the world are working on different aspects related to the ultimate goal of producing transgenic mosquitoes for disease control. The state of art in these technologies and the expected research direction in India will be presented.

14

A NOVEL HOMEODOMAIN PROTEIN ENCODING GENE ASSOCIATED WITH PNS IN *DROSOPHILA* IS ALSO PRESENT IN *ANOPHELES STEPHENSI*

Dileep N. Deobagkar

Molecular Biology Research Laboratory, Department of Zoology, University of Pune, Pune 411 007

As a part of investigations on molecular analysis of growth and differentiation of the malarial vector mosquito *Anopheles stephensi*, we identified a 1.7 kbp DNA sequence of *Drosophila* (EMBL Accession No. Z 29571) to contain significant homology to the *Anopheles stephensi* DNA. This sequence harbours an ORF complete with the Promoter, a consensus initiator codon, tandem termination codons followed by a poly A signal. This 122 amino acid sequence does not have homology to any reported sequence do for in all databases. In absence of such information, functional genomics approach was used to predict that this 122 amino acid protein is a homeobox protein, which could interact with an *engrailed* binding DNA sequence. This was confirmed in a DNA gel retardation assay. The corresponding 366 nucleotide long RNA hybridized at 2 locations on right arm of 2nd chromosome in ovarian nurse cells polytene chromosome spread of *Anopheles stephensi*. This sequence has been found to occur in other Dipterans and also in mammals. its expression was highest in pupae whereas in the *Drosophila* embryos, the homologous transcript could be located in PNS related cells in stage 11-13 embryos.

15

CONTROL OF SOME INSECT VECTORS OF PARASITIC DISEASES

M.S. Jagannath
Centre of Advanced Studies, Department of Parasitology, Veterinary College, University of Agricultural Sciences, Bangalore 560 024

The role of insects in transmission of parasitic diseases and the mechanisms by which they transmit parasites has been elucidated. The factors which help in the dissemination and establishment of diseases indicate that vectors in the strict sense are definitive hosts for parasites. Parasitism by arthropods places them in frequent intimate and often dependent association with vertebrates.

The role of beetles, tabanid flies, culicoides, simuild and muscid flies in the transmission of parasitic diseases will be highlighted. The different methods of control of these vectors including managemental, chemical and biological will be reviewed.

The factors which pose difficulties in the control and implementation of these measures in the field will be discussed.

CONTRIBUTED PAPERS - ORAL

16

DEVELOPMENT OF RESISTANT STRAIN OF *LEISHMANIA DONOVANI* IN HAMSTERS

Anuradha Dube, S. Sundar* and J.C. Katiyar
Division of Parasitology, Central Drug Research Institute, Lucknow 226 001 and
** Department of Medicine, IMS, Banaras Hindu University, Varanasi 221 005*

The treatment for a patient infected with leishmaniasis is based on antimonials which are still the drugs of choice despite their cardiac and renal toxicity, difficulty of administration and high costs. Second line drugs, such as pentamidine and amphotericin B, do not have a therapeutic index as favourable as that of pentavalent antimonials, they also require long term therapy and often induce toxic effects. The absence of a range of effective drugs against parasitic protozoa is exacerbated by the development of drug resistance in parasites. Leishmania infections that are refractory to antimonial chemotherapy have been recognized since the early 1940s and are increasing world wide. The development, *in vitro* of drug resistant parasite cell lines has been instrumental in our understanding of the mechanism of drug resistance in parasitic protozoans. Similarly development of *in vivo* model in rodents will further give better understanding of drug behaviour in the host as well as host-parasite relationship. Moreover screening of newer synthetic compounds or plant extracts against drug resistant parasite may result into a development of a suitable drug against the resistant parasite.

With this aim we collected few clinical isolates of Sbv resistant parasite from the field and maintained them *in vitro* in promastigote form. These promastigotes were infected intracardially into naive hamsters. The infection establishes by day 30 p.i. and is fatal by the end of 2 to 2-1/2 months p.i. The infected hamsters were found resistant to the therapy of sodium stibogluconate at 20 and 10 mg/kg for 5 days.

17

STUDY ON BREEDING HABITATS AND DENSITY OF IMMATURE STAGES OF SANDFLIES OF IMPLEMENTING BIOLOGICAL CONTROLS

S. Kesari, V. Kumar, A. Palit, K.Kishore, M.S.Roy and S.K.Kar
Rajendra Memorial Research Institute of Medical Sciences, Indian Council of Medical Research, Agamkuan, Patna 800 007

The knowledge of breeding habitats of sandflies is very important to work out the peak season of sandflies abundence in relation to temperature and humidity which in turn, will help to evaluate the appropriate time of chemical control measures particularly against the vector species of Kala-azar *P.argentipes*. Simulteneously, this can also be of immense value in corre-

lating adult population in its natural habit and immature stages in the soil.

The exact breeding sites of most of the species of sandflies remain unknown. The importance of knowing more about the breeding habitats of vector sandflies for their control was first realised by Smith et. al. who made an unsuccessful attempt to control *P.argentipes* by antilarval measures. It was reported that sandfly immature could be controlled by treating potential breeding place with 15-20% solution of chloride of lime. Recent studies by other workers indicated that mud with lime plastering of walls and minor engineering works in human dwellings and cattlesheds could considerably reduce the adult population of *P.argentipes*. They attributed this to the filling up of creek and crevices on the wall that they considered as the major breeding sites and the larvicidal effect of lime. But since creek and crevices are the resting sites of adults, less number caught in the resting site collection might be due to destruction of these resting sites. However, other studies indicated that sandflies could be controlled either by selective treatment with insecticides or destruction of breeding habitats.

In the present study, 230 soil samples collected from different possible breeding sites in an endemic village in human dwellings and cattlesheds. Out of which 115 samples were examined microscopically and in two samples we found two live 3rd instat larvae and other 115 sugar floatation technique in which 20 numbers of pupal excuvae were found.

The live larvae were kept in the insectorium for further rearing keeping temperature and humidity as desired. After due time the larvae moulted to pupae and ultimately emerged into female *P.argentipes*.

The soil sample collected for this pupae were also examined for its chemical and salt composition.

The study reveal that even in the winter months the larvae remain in the soil in diapause condition. It is only the environmental conditions i.e. temperature and humidity, which play an important role in propagation of sandflies' life cycle.

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BIOLOGICAL CONTROL USING *GAMBUSIA AFFINIS* AS AN ADJUNCT METHODOLOGY FOR CONTROL OF MALARIA AT VELLORE IN TAMILNADU - AN ASSESSMENT REPORT

V.Rajagopal* Narayanasamy and N.C.Appavoo*****

** Zonal Entomological Team, 23, Vepanana Street, Velapedi, Vellore 632 001, T.N.*

*** Municipality of Vellore.*

**** Directorate of Public Health and Preventive Medicine, Chennai*

A Study to assess the role of *Gabusia affinis* in the biological control of malaria as the adjunct methodology at Urban Malaria Scheme area of Vellore Town in Tamil Nadu was conducted in 1998.

With a population of about two lakhs, Vellore Town is the District Headquarters and also the main commercial central District Vellore of Tamilnadu.

12 numbers of Municipal wards among the total 48 wards were identiified as malaria high risk wards. *Anopheles stephensi* is the principle vector in the transmission of malaria. Indigenous transmission of *P. falciparum* is unknown at Vellore.

Initial entomological surveys indicated predominant vector breeding in the wells. 538 wells in all the malaria high risk wards and neighbouring wards were brought under biological control using *Gambusia affinis* from 1995 onwards. Judicious replenishments were periodically undertaken with community participation.

Malaria situation at the end of the year 1998 indicated a significant 85% reduction in the indigenous malaria cases when compared to the situation during 1995.

Detailed analysis of this adjunct methodology of control of malaria has been discussed in relation to the epidemiological situation, vulnerability of the area and the conventional strategies during the study period.

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STRATEGY FOR VECTOR INCRIMINATION

WAJIHULLAH
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202 001 U.P.

Malaria, one of the six diseases included in WHO special programme re-emerged in several states in India and took hundreds of lives in preceding years and this year too. Falciparum malaria predominated in foothills, forests and forest fringes, especially in tribal settlements where man-mosquito contact is maximum because of poor housing, poor clothing and exposure during work in jungles. Presence of specific breeding sites of vector species in vicinity make situation more conducive for malaria transmission. Sharma (1996) indicated that tribal population alone contributes about 30% of malaria cases and 75% deaths. Present study was carried out in tribal population of Sonapur, one of the endemic pockets of malaria in Kamrup District, Assam where 80% of the cases were of *Plasmodium falciparum.*

In india more than 50 Anophelines are operating but not more than 7 or 8 are responsible for malaria transmission. Therefore, for effective malaria control, ecology and biology of vector must be known. For knowing suspected vectors, one must concentrate on anophelines resting indoor, followed by blood meal analysis in order to ensure whether they are anthropophilic, zoophillic or both. This helps in shortlisting the suspected vectors. During the present study efforts were made to find out human biting index and sporozoite positivity rate. Problems faced during vector incrimination will also be discussed. Suspected vectors were collected from indoor restings and their blood meal was analysed by ELISA technique. Dissections were performed only on anthropophilic species to see the gland positivity of sporozoites. *Anopheles minimus* happened to be the principal vector responsible for malaria transmission in Kamrup District, Assam. *Anopheles balabacencis (A. dirus)* which too is a anthropophilic species, probably helps in malaria transmission in dense forested areas.

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ANALYSIS OF VARIOUS CHARACTERS MEANT FOR CHARACTERIZATION OF CESTODE SPECIES REFERABLE TO GENERA - *RAILLIETINA* (*RAILLIETINA*) FUHRMANN, 1920 AND *ROSTELUGNIA* SPASSKII, 1984

Sarbjit singh grewal and Satwinder Kaur
Department of Zoology, Punjabi University, Patiala 147 002

As many as 512 Columbiformes birds have been sacrificed during the course of present investigation. The cestode infection leading to the recovery of 4070 cestodes of different genera have been recorded from 357 birds. Out of 1157 permanent cestode preparations, 726 belong to *Raillietina (Raillietina)* and 191 to *Rostelugnia,* while the remaining pertain to some other genera.

One of the significant inference drawn from the study reveals that all the cestodes of a genus recovered from a single host specimen represent single species with overlapping characteristics, provided there are sufficient number of cestodes available. Upon analysis of various morphometric and meristic characters, it has been analysed that all of them are not taxonomically important. In the genus *Raillietina*, the most significant characteristics for species identification are : length of the rostellar hooks and testes number followed by width of scolex, sucker diameter, width of rostellum, size of cirrus sac and finally diameter of eggs. While in the case of *Rostelugnia*, the species identification characteristics are: testes in one or two groups, extension of cirrus sac, length of rostellar hooks, testes number and lastly diameter of rostellum and sucker, taken together.

Whereas, the present intensive and extensive study helps in proper delimitation of different species and also helps in checking the menace of multiplicity of species on one or the other pretext.

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SEASONAL PREVALENCE OF *CULEX QUINQUEFASCIATUS* AND VECTOR FILARIAL INFECTION AT RAIPUR CITY

A.K.Gupta, Vandana Dixit and G.B.K.S. Prasad
School of Life Sciences, Pt. Ravishankar shukla University, Raipur, M.P and SOS Biochemistry, Jiwaji University, Gwalior 492 010, M.P.

Culex quinquefasciatus is the main vector of bancroftian filariasis in India. The transmission dynamics of filariasis is profoundly influenced by bionomics of the vector in a given geographical region. A study was undertaken to determine the monthly variations in the

density distribution of *Cx. quinquefasciatus* and the vector infection status at Raipur city. A total of 3976 mosquitoes were collected from six selected spots of Raipur city between October 95 to September 96. The highest density (163/man h) was recorded in the month of December followed by November and October. The lowest density recorded was in the months of June and May (15-20/man h). There appears to be a significant correlation between minimum temperature and density pattern of the vector. Analysis of spot specific densities indicate that the highest density (77/man h) of mosquitoes was at Budhapara and the lowest (39/man h) was at Fafadih. The December month recorded highest density in almost all the study spots. A vector infection rate of 4% was recorded in the study region and it appears to have no relationship with density variations of the vector. A vector infectivity rate of 0.25% was recorded and it shows no relationship either with infection rate or density variations of the vector. The highest vector infectivity rates was recorded at Tikarapara, Kankalipara and Rajatalab. The infectivity rate was found to be zero at Budhapara which recorded the highest density of the vector population. The host effciency rate was found maximum (0.14) at Kankalipara and it appears to have no relationship with infection rate. The host efficiency was found very high in January and February months as compared to rest of the period.

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STUDIES ON HISTOPATHOLOGY OF SNAIL VECTORS WITH REFERENCE TO CERCARIAL INFECTIONS

C. Vijaya Lakshmi and Ch. Dhanum Kumari
Department of Zoology, Andhra University, Vishakhapatnam 530 003

Cerithacean snails of *Thiara tuberculata* and *Cerithidea cingulata* (Order: Gastropoda) act as vectors for various digenetic trematodes infecting economically important fishes and in some instances even man. *Thiara tuberculata* is a habitant of freshwaters, whereas *C.cingulata* is of brackishwater localities. In this paper the pathological effect of larval stages of *Acanthoparyphium* sp. and *Centrocestus formosanus* on the hepatopancreas of the vectors *T. tuberculata* and *C. cingulata* respectively have been studied. The intramolluscan stages of *Acanthoparyphium* sp. are redial and *C. formosanus* are sporocysts. The histopathological changes associated with these infections on the snails have been studied. Some experiments have been conducted in relation to the parasitic activity and host response of the snail and these changes are also incorporated in the present paper.

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EXPRESSION OF THE RIBOSOMAL PHOSPHO PROTEIN ON THE SURFACE OF PARASITE AND OTHER CELLS

Subhash Singh, Alfica Sehgal and Shobhona Sharma
Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhaba Road, Mumbai 400 005

A differential immunoscreen carried out earlier in the laboratory identified several expression cDNA clones of *Plasmodium falciparum*, which reacted exclusively and extensively with the immune sera. The clone, which reacted with 87% of immune samples, was identified as *P.falciparum* homologue of the ribosomal phosphoprotein, PO. Antibodies generated against the protein can lead to inhibition of the parasite growth in culture, specifically at the stage of erythrocyte invasion (Goswami et al., 1997, *J Biol. Chem.* **272** : 12138).

Antibodies against the protein have been used in indirect Immuno Flourescence Assay (IFA) carried out on different cell types. The assay indicates presence of this protein on every merozoite surface. Mammalian cell lines such as CHO, K562, Daudi, U937; peripheral human blood cells and yeast cells were also tested by similar assay for the presence of PO on the surface. IFA reactivity was seen on all the cell lines and yeast although only in a subset of cells. However, the peripheral human blood cells showed no IFA reactivity. Western analysis done with crude extracts of *Plasmodium*, some of the cell lines and yeast cells, showed a band around 38-39kDa, which is the molecular weight of the PO protein. It has been reported earlier that PO plays a vital role in the ribosomal assembly using knock out mutants in yeast (Santos and Ballesta, 1994 *J Biol, Chem.* **269** 15689-1569). These results, therefore, suggest a dual role of the PO protein, in ribosomes and yet another novel function associated with its surface localization.

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STAGE SPECIFIC EXPRESSION OF GENES IN *PLASMODIUM FALCIPARUM* : A TOOL FOR RATIONAL DRUG DESIGN

Vidya Prasanna Kumar, Suryaprakash, H. Jayashree, Suresh Solapure and Santanu Datta
Astra Biochemicals (P) Ltd., 18th Cross, Malleswaram, Bangalore 560 003

The malarial parasite *P. falciparum* go through a complex set of developmental growth pattern during its passage from mosquito to human. Even in the simplified *in vitro* culture which parallels the erythrocytic stage of growth in human, it goes through stages described as rings, trophozoites and schizonts. That these stages have different metabolic requirement is well documented. While screening for antimalarial compounds in the *in vitro* culture not much

attention is paid to the stages of growth. Unless specific techniques are adopted, the parasites in the *in vitro* culture are generally a randomized mixture of all stages of the parasites. In contrast, in *P. falciparum* infected human the parasites show a synchrony in its growth pattern. Recent studies indicate that antimalarial drugs in general show stage specific inhibition pattern in *in vitro* parasite culture. If drugs act as enzyme inhibitors, which in general they are, then one could argue that the stage specific action of drug are the result of stage specific expression levels of the target enzyme. It is thus imperative that when rational drug design is attempted on the basis of inhibition of a certain key enzyme, the relative level of the enzyme on various stages of growth be monitored and be correlated with *in vivo* inhibition of growth. It is a generally accepted concept that the level of poly adenylated mRNA corresponds to the relative level of its translated product. We have used this concept to monitor the stage specific expression of a battery of genes (HGPRT, LDH, TPI, Falcipain, Plasmepsin II, Actin, DHFR-TS) in the parasite *P. falciparum* using a semiquantitative RT-PCR technique. Specific inhibitors to some of these gene products were used to monitor the stage specific inhibition of the parasite growth. Results correlating the stage specific expression of these genes to the stage specific growth inhibitions are discussed.

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LACK OF ASSOCIATION BETWEEN CHLOROQUINE RESISTANCE AND ALLELIC VARIATION OF *pfmdr 1* GENE IN *PLASMODIUM FALCIPARUM* ISOLATES FROM INDIA

Pramatha R Bhattacharya and C. Radhakrishna Pillai
Malaria Research Centre,22, Sham Nath Marg, Delhi 110 054

Prevalence of chloroquine resistant *Plasmodium falciparum* has made the control of falciparum malaria more complicated. Point mutations at nucleotide positions 754, 1049, 3598, 3622 and 4234 in *pfmdr 1* gene considered to be associated with chloroquine resistance found to be controversial. We, therefore tested 18 chloroquine sensitive and 22 resistant isolates from India to further examine the role of *pfmdr 1* gene in chloroquine resistance. We failed to correlate allelic variation of *pfmdr 1* gene and chloroquine resistance in Indian *P. falciparum* isolates.

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PREVALENCE OF MALARIA VECTORS IN DIFFERENT ECOTYPES ALONG THE BRAHMAPUTRA VALLEY OF ASSAM AND THEIR DISEASE TRANSMISSION PATTERN

P. Dutta, S.A. Khan, A.M. Khan, C.K. Sarma and J. Mahanta
Regional Medical Rearch Centre, NE Region, (ICMR), P.O. Box # 105, Dibrugarh 786 001 (Assam)

Entomological surveys conducted in lower, middle and upper parts of Assam covering different ecotypes prevailing in this region reveal that the Anopheline fauna is very rich in foot hill and forest ecosystems where *Anopheles dirus* and *An. minimus* have been incriminated as malaria vectors. Due to preponderance of these vectors, these areas are found to be highly malarious particularly falciparum malaria (comprising 70-97 % of the total cases). In other areas namely, plains, river-island and tea gardens situated in plains, malaria is not a problem due to non exixtence of these vector species. In longitudinal studies conducted in two different areas, one in middle part of Assam (Karbi Anglong District) and another in Dibrugarh District of upper Assam, it is observed that *An. minimus* is prevalent in ecotone zones closer to foot-hills with streams and paddy fields transmitting malaria all throughout the year (SPR ranging from 13.5 - 20.0) and shows high vector density during June to September. In contrast, *An. dirus* occupies forest and forest fringes and transmission of malaria is found to be seasonal mostly during the period of September - November with high vector density for the period from June to October. Malaria epidemiology, therefore, in the two different ecosystems with the prevalence of specific vector species is found to be different.

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SOIL-TRANSMITTED HELMINTHIASIS AS A CAMOUFLAGE IN SECONDARY DISEASE MANIFESTATIONS

Indrani Paul
Calcutta School of Tropical Medicine & Department of Zoology, University of Calcutta 700 029

Soil-transmitted helminth infections remain one of the major public health hazards of India. Illiteracy, poverty, lack of sanitation facilities, improper primary healthcare all seem to add to the multitude of problems that these nematode infections seem to represent.

A longitudinal study was conducted during August 1993 - August 1994, in an urban slum of Visakhapatnam, Andhra Pradesh, South India. All individuals were children between the age group of 7 - 13 years below the poverty line with parental occupation representing fishing or waged labour.

The study estimated the prevalence and intensity profile together with haematological assessment of certain basic parameters like WBC, hemoglobin level, total protein estimations. Single stool specimens were collected and evaluated for helminth infections by formalin-ether sedimentation technique. Pre-treatment observations showed that ova count and haematological levels were on the higher side. Each child was administered a single dose of 400mg of albendazole for expulsion of worms and post-treatment data was collected at regular intervals to study the pattern of infection, reinfection dynamics and blood parameters over time.

Prevalence and intensity were of moderate range and *Ascaris* worm burden was low. Soil-transmitted nematode infections seem to cause an elevated response in the haematological profile with gradual down regulation over time following drug intervention ($p < 0.05$). The results will be discussed on different aspects including socio-economic, dietary and environmental.

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MALARIA TRANSMISSION DYNAMICS IN AND AROUND NANAK MATTA DAM UNDER CHANGING ECOLOGICAL SCENARIO

R.P. Shukla, S.N. Sharma, V.K. Kohli and V.P. Sharma*

Malaria Research Centre, Field station, Inderjeet Garden, Bhotia Parao, Haldwani 263 141

* *Malaria Research Centre, 20, Madhuvan, Vikas Marg, Delhi 110 092*

To study transmission dynamics of malaria, entomological and parasitological surveys were made in 3 different ecotypes viz., watershed (forest) of Bhabar, District Nainital and seepage (dam) and plain (non-forest and non-dam) of Terai, District Udham Singh Nagar from July 1996 to June 1997. the two malaria vectors i.e., *Anopheles culicifacies* and *A. fluviatilis* and 6 other anophelines were found breeding in various habitats. Adult densities of *A. fluviatilis* was recorded during post monsoon and spring seasons, whereas prevalence of *A. culicifacies* was found during pre-monsoon and monsoon periods in all ecotypes. *A. fluviatilis* had higest prevalence in forest ecotype. Proportion of fully fed females was higher as compared to semigravid which indicated exophilic tendency of vector species. Night biting rate using human bait revealed higher rate in outdoor as compared to indoor for both the vector species. No human biting was found in dam ecotype. In plain area biting of *A. fluviatilis* was recorded during first and 4th quarters of the night. In forest ecotype, both *A. fluviatilis* and *A. culicifacies* were found biting human bait. Parity in *A. fluviatilis* by ovariole dilatations revealed high rate in forest and plain as compared to dam ecotype. A total of 989 *A. fluviatilis* and 193 *A. culicifacies* were dissected for vector incrimination study. Natural infection in gland was found in one specimen of *A. fluviatilis* with 0.1% sporozoite rate in November 1996 from forest ecotype only. No infection in *A. culicifacies* was found from either of the ecotypes.

Various epidemiological parameters viz ., SPR, SFR, API and ABER revealed 48.5, 35.7, 245.8 and 50.7 in forest, 1.8, 0, 1.2 and 6.4 in dam and only 4.8 % ABER in dam ecotypes. The present findings revealed that *A. fluviatilis* is playing active role in malaria transmission in forest ecotype.

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BIO-ENVIRONMENTAL CONTROL OF MALARIA - THE KARNATAKA EXPERIENCE

S.K. Ghosh[1], S.N. Tiwari[1], A.K. Kulshrestha[1], T.R.R. Sampath[1], T.S Sathyanarayan[1], V.P. Sharma[2], M.V. Murugendrappa[3] and S.K. Subbarao[2].

1. Malaria Research Centre, Field Station, E.D. Hospital Campus, Bangalore 560 038

2. Malaria Research Centre, 22 - Sham Nath Marg, Delhi 110 054

3. Directorate of Health Services, Ananda Rao Circle, Bangalore 560 009

Karnataka contributes about 7% of malaria in India. As such malaria is unstable in this State. However, in some areas this disease is endemic. Bio-environmental control of malaria was successfully demonstrated in District Kolar and subsequently in District Hassan. Geographical Reconnaissance carried out in these areas revealed that wells are the major breeding sites for the vector mosquito *Anopheles culicifacies*. Accordingly larvivorous fish Guppy (*Lebistes reticulatus*) were released in all the wells. A great reduction on malaria incidence is observed. In PHC Kamasamudram, District Kolar API came down from 41.4 in 1993 to 1.3 in 1998; while in PHC Banavara, District Hassan where only fish was used API came down from 154 in 1995 to 1.8 in 1998. In another PHC Kanakatte three groups of villages were taken. These were villages with i) Cyfluthrin impregnated bed nets area only. In this area API came down from 86 in 1995 to nil in 1998, ii) Area with both impregnated bed nets and fish. In 1995 API was 187.17 which came down to 0.2 in 1998 and iii) Guppy fishes only. In this area API was 77.63 in 1995 which came down to 0.1 in 1998. Hence, the use of larvivorous fishes in such eco-climatic areas would be an effective control agent.

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PCR AMPLIFICATION KINESIN GENE FOR THE ACCURATE DIAGNOSIS OF KALA-AZAR CAUSED BY DIFFERENT CLINICAL ISOLATES OF *LEISHMANIA DONOVANI*

R. Sivakumar, Saba Shahdad, D.P. Mohapatra and Sarman Singh
Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi 110 029

Kala-azar is one of the endemic parasitic infections in the Indian sub-continent, caused by a kinetoplastid protozoan parasite *Leishmania donovani*. The disease claims serveral thousand lives every year in Bihar and West Bengal in particular. For the diagnosis of kala-azar, bone marrow or splenic puncture aspirates are examined microscopically to demonstrate the parasitic infection. These techniques are invasive, painful and some times fatal. Serological diagnosis of kala-azar is also being applied in more and more centres. Various PCR based diagnostic methods are also reported for the detection of parasites in the blood, bone marrow and splenic aspirates using amplification of kinetoplast minicircle conserved and variable DNA sequences. But none of the detection method can be used as strain specific tool. Thus, we developed a PCR based method to diagnose the infection caused by different strains of the parasite using a new primer set for the amplification of a repetitive sequence of the kinesin gene in the *Leishmania* genome. The PCR products were in the form of a ladder of amplicons. The number of bands depends upon the causative strains of the parasite. The results indicated the presence of strain specific number of repeats of kinesin gene in their genome. The sequence of each repeat is now being analysed. The method was standardised using the DNA from different strains of the parasite maintained in *in vitro* culture system. Efforts are on to apply this PCR method on the patient samples for specific diagnosis. This method may be used for carying out molecular epidemiological studies directly from the clinical samples.

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CHARACTERIZATION OF A 95 k da *PLASMODIUM FALCIPARUM* ANTIGEN EXPRESSED ON THE INFECTED ERYTHROCYTE MEMBRANE

Mrinal K Bhattacharyya, Subhash Singh, Tirtha Chakraborty and Shobhana Sharma
Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005

A cDNA expression clone of the human malarial parasite *Plasmodium falciparum*, λPf2, was found to react exclusively with malaria immune sera (Lobo et al, Mol. Biochem. Parasitol. 1994, 68, 151-154). The 247-bp cDNA insert of λPf2 was in frame with *lacZ*, with an open

reading frame of 43 amino acids. From the predicted open reading frame, an olio-peptide was synthesized and conjugated with BSA. Rabbit antibodies were raised against this conjugated protein. This anti-peptide antibody recognizes the λPf2 expression clone and lit up a 95 kDa *P. falciparum* protein on Western blots. Immunoflourescence studies on permeablized as well as non-permeablized *P. falciparum* infected erythrocytes showed the presence of Pf2 protein on the surface of the infected erythrocytes at the trophozoite and schizont stages. This finding was further confirmed by antibody mediated agglutination assay. In a complement dependent pathway, the anti-peptide antisera inhibited the growth of *P. falciparum in vitro*, thus indicating a protective nature of the Pf2 epitope. Immunoflourescence results show that this protein is present on the surface of red cells from other Plasmodial species. All these results indicate that Pf2 is a novel and conserved protein of *Plasmodium falciparum*.

Primers generated for the Pf2 clone could pick up a clone from a Yac genomic library of *P. falciparum*. Subcloning of the Pf2 gene from this clone is in progress and should provide more information about the gene sequence and function. Data with respect to Pf2 gene and some aspects of its function will be presented.

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SIMPLE ASSAYS IN THE DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

S.C. Parija
Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Pondicherry 605 006

Laboratory diagnosis of cystic echinococcosis (CE) in the field or rural health center catering to the needs of rural population, especially in the developing countries is met with many difficulties. This talk reviews the need for simple and rapid immunoassays for the diagnosis of CE in such conditions. Recent developments in the development of simple immunoassays in serology of CE includes the demonstration of (a) hydatid antibodies in the serum by Dot-enzyme-linked immunosorbent assay (Dot-ELISA), Rapid - indirect haemagglutination test (Rapid-IHA), Latex agglutination test (LAT) and Hydatid-antigen dot binding immunoassay (HA-DIA) ; and (b) hydatid antigen in the serum by Co-agglutination (Co-A) and Dot-ELISA. Demonstration of hydatid antigen in urine from the patients with CE by Counter-current immunoelectrophoresis (CIEP) and Co-A is a noted advancement in the serology of CE. Also, detection of hydatid antigen in cystic fluid as an alternate to fluid microscope is a recent approach to diagnose hydatid aetilogy of suspected hydatid cyst by simple assays such as CIEP and Co-A. These simple assays which have the potential for wider use in the field or poorly equipped laboratories for serodiagnosis of CE are discussed.

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HISTOPATHOLOGICAL STUDIES ON ANOPLOCEPHALINE CESTODE, *MONIEZIA (BLANCHARIEZIA) KALAWATISP.NOV.* INFECTING *CAPRA HIRCUS* L.

Sanjay Nanware, Baba Jadhav* and Mohan Babare

Post Graduate Department of Zoology, Arts, Science & Commerce College, Naldurg 413 602, Tq. Tuljapur, Dist Osmanabad, M.S.

** University Department of Zoology, Dr. B.A. Marathwada University, Aurangabad 431 004. M.S.*

Host-parasite relationship results in gain of one organism and loss of another. It leads to various diseases and disorders. Significant findings are seen with depression in leucocyte and weight gain. When host-parasite equilibrium is disturbed, serious disease or epizootics in host is the consequence, resulting in depletion or weakening of host. The study pertains to histopathological observations of *Moniezia (Blanchariezia) kalawati* and predominant intestinal anoplocephalidean cestode of host *Capra hircus*. It was observed that the worm *Moniezia(Blanchariezia) kalawati* attaches and entagles, invades in intestinal villi, it inflicts injuries to tissues; necrosis of tissue due to pressure of parasites which of common occurrence. Inflammation in intestinal tissue at sites of attachment was observed. In T.S. of intestinal tissue, worm attached to mucosa and sub-mucosa, and slowly invades host tissue. Parasites are seen either free in intestinal lumen or mixed with necrotic debris consisting of mucosal epithelium and mononuclear cells, or embedded in intestinal mucosa with their anterior end. Submucosa exhibited fibrocellular reaction consisting of fibrocytes, lymphocytes, plasma cells and macrophases. The level of pH in intestine is 9, this alkalinity seems to be quite favourable for the cestode *Moniezia (Blanchariezia) kalawati* for proteins, fats and glucose content in the lumen is rich and so the worm finds it easy to be absorbed, through integument for nourishment and growth. The integument, longitudinal muscles and mature proglottids have much reserve deposits and it is clear that worm absorbs these material from host tissue. In T.S. of intestinal tisue, it has been observed that, intestinal villi encircles the scolex of worm and in some intestinal villi tissue get disturbed by invasion of the worm scolex deeply, so it can be concluded from the above and by histochemical studies, that worm absorbs glycogen, protein and lipid from host tissue causing damage to hosts intestinal tissue.

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PLASMODIUM YOELII YOELII INFECTION INDUCED CHANGES IN SALIVARY GLAND PROTEINS OF MALARIA VECTOR *ANOPHELES STEPHENSI* (DIPTERA : CULICIDAE)

Harish Shandilya, Vrinda Yadav* and S. K. Gakhar
Department of Biosciences, Maharshi Dayanand University, Rohtak 124 001, Haryana

Parasitism by *Plasmodium yoelii yoelii* induced changes in the qualitative and quantitative pattern of soluble proteins of salivary glands in ageing malaria vector *Anopheles stephensi* have been studied by SDS-PAGE.

In total, 18 polypeptides were found to be induced in the salivary glands by infected blood meal during various stages of adult life. One low molecular weight polypeptide, 30 KDa was present in all the infected stages. In the oocysts positive stage, no new polypeptide could be found n the salivary glands. Five low molecular weight polypeptides (14.4,21,23,63 & 65 KDa) and 5 high molecular weight polypeptides (72, 82,142,151 & 153 KDa) were induced in the 11 days post blood feeding stage which coincided with the invasion of sporozoites in the salivary glands. However, 5 low molecular weight polypeptides (19.5, 29, 31, 34 & 66 KDa) did not express in the salivary glands due to infected blood meal.

Quantitatively, soluble proteins decreased by about 3 times in the salivary glands after blood meal. The amount of soluble proteins further depeleted by about 2.5.% in the salivary glands due to infected blood meal at oocysts positive stage and by about 26% in sporozoites infected mosquitoes. The changes in polypeptides in infected mosquitoes. The changes in polypeptides in infected mosquitoes will be discussed in relation to enhancing the parasite transmission potential.

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CHANGES IN POLYPEPTIDES IN RELATION TO FECUNDITY REDUCTION IN *PLASMODIUM YOELII YOELII* INFECTED MALARIA VECTOR *ANOPHELES STEPHENSI* (DIPTERA : INSECTA)

S.K. Gakhar and Harish Shandilya
Devlopment Biology & Biotechnology Laboratory, Deptartment of Biosciences, Maharshi Dayanand University, Rohtak 124 001, Haryana

The qualitative and quantitative analyses of soluble proteins of haemolymph and ovaries during the development and ageing of *Anopheles stephensi* in uninfected and infected by *Plasmodium yoelii yoelii* has been carried out by SDS-PAGE. Fecundity reduction was

observed in *Plasomodium yoelii yoelii* infected mosquitoes. The changes in polypeptides pattern during haematophagy, sugar feeding have also been studied in ageing mosquitoes.

Results indicated stage-and tissue-specific induction of soluble proteins when the mosquitoes were fed on malarial parasite *Plasmodium y. yoelii* infected mice. It has been suggested that parasite induced 18 haemolymph proteins could provide protection to cells against the parasite induced damage and its synthesis may be considered as desirable feature in conferring parasite tolerance to the adult.

The disappearance of 7 polypeptides (19, 30, 35, 54, 61, 73 & 121 kDa) and reduced expression of 6 other polypeptides in the ovaries could be ascribed to reduced fecundity in *P. y. yoelii* infected mosquiotes. The quantity of soluble proteins was depleted significantly due to parasite infection i.e., in haemolymph (12%) and in ovaries (28%).

The possible role of these polypeptides particularly during parasitism and in relation to fecundity reduction during three gonotrophic cycles will be discussed.

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PARASITIC INFECTION IN LABORATORY RATS (*RATTUS NORVEGICUS*) AND ITS EFFECTIVE MANAGEMENT

D.C. Purohit
Animal facility, Industrical Toxicology Research Centre, Lucknow

Like man and other domestic animals, the laboratory animals have been found to serve as host for wide variety of protozoan and metazoan parasites. Colony bred wistar rats (*Rattus norvegicus*) were screened for parasitic infection and positive animals, on the basis of faecal examination, were separated from breeding stock. These animals were kept in 4 groups (12-15 animals in each group) on normal pelleted diet and water ad lib. During the study it has been found that these animals were infected with either single or mixed infection with *Syphacia muris; S. ovelata ; Aspeculuris tetraptera* and some species of enteric protozoans. Group I was treated orally with albendazole; group II with piperazine ; group III with combination of albendazole and piperazine and grouip IV with metrozyle respectively at recommended dose level. The treatment of each drug to the respective grouip was continued up to 5 days and simultaneously the faeces of all the animals were examined for ova/cyst of enteric parasites till their disappearance. The results suggest that single dose of any drug is not sufficient to eliminate the parasite from the gut, observations also reveals that the combination of albendazole and piperazine were significantly effective in management of helminthic infection in laboratory rats.

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IMPROVED METHODS FOR SURVEILLANCE OF VIRUS INFECTIONS IN VECTORS OF JAPANESE ENCEPHALITIS AND DENGUE

A. Gajanana

Centre for Research in Medical Entomology, 4, Sarojini Street, Chinna Chokkikulam, Madurai 625 002

Japanese Encephalitis (JE) and Dengue (DEN) are important mosquito-transmitted virus diseases in India. Several severe outbreaks of JE and DEN have occurred in many parts of the country. In India, vectors of JE belong to *Culex vishnui* group and vector of DEN is *Aedes aegypti*. A sound surveillance system is needed to forecast epidemics and take preventive control measures. Monitoring virus infection in vector mosquitoes, forms an intergral component of such a surveillance system. This involves screening of large numbers of wild-caught mosquitoes for virus infections in endemic areas in different seasons over a long period. Classical tissue culture and suckling mouse inoculation techniques are not suitable for screening a large sample size as the techiniques are time consuming, cumbersome and less sensitive. Hence, a rapid and sensitive Enzyme Immunoassay (EIA) for detection of virus infections in wild caught mosquitoes has been improved for JE vectors and developed for dengue vectors for field use. Several thousand pools of field-collected mosquito vectors of JE and dengue have been tested by EIA in conjunction with insect bioassay (*Toxorhynchites splendens* inoculation followed by IFA) for detection and isolation of viruses.

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IMMUNE RESPONSE TO NON PARASITE VACCINE ANTIGENS IN HUMAN LYMPHATIC FILARIASIS

Subas, P. Kaliraj, Kunthala Jayaraman, Thomas B. Nutman*, and R.B. Narayanan

Centre for Biotechnology, Anna University, Chennai 600 025

** Laboratory of Parasitic Diseases, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA*

Human Lymphatic Filariasis caused by the nematode parasites *Wuchereria bancrofti* and *Brugia malayi* is associated with cellular responses to specific parasite antigens characterized *by poor lymphocyte proliferation, impaired production of T helper cell type (Th1) cytokines and a relatively enhanced production of Th2 cytokines*. This antigen specific T cell hyporesponsiveness may also extend to the cellular responses to other parasite antigens. Since individuals living in endemic areas are infected with multiple helminth infections, the investigation of the relationship between human helminth infections and the immune response to non-

parasite antigens is of great public health significance for a variety of reasons. For instance, if pre-existing infections can influence immune responses against other antigens, the implications for the effectiveness of vaccination programs can be quite significant especially in developing countries.

In this study, an attempt is made to understand the effect of concurrent active bancroftian filarial infection on the immune response to non-parasite vaccine antigens following vaccination with routinely administered and commercially available vacciness such as *Hepatitis B, Tetanus toxoid, and Typhim Vi*. The proliferative cytokine and antibody responses to the above non-parasite vaccine antigens before and after 1,3, and 6 months post vaccination is being investigated in individuals with circulating microfilariae, patients with chronic lymphatic obstruction, and exposed but unifected individuals. Further, the levels of circulating antigens that serve as a marker for adult worm burden is also monitored. The results of this study will be presented.

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VACCINATION WITH SINGLE DOSE OF ALUM PRECIPITATED ALM+BCG AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS : A PRELIMINARY REPORT

Bindu Srivastava, Alka Misra, Preeti Sharma, J.K. Srivastava, Anuradha Dube, J.C. Katiyar and Sita Naik*

Division of Parastiology, Central Drug Research Institute, Lucknow 226 001

** Department of Immunology, S.G.P.G.I., Lucknow*

Autoclaved Leishmania Major (ALM), a WHO vaccine under phase II clinical trial against cutaneous leishmaniasis along with BCG showed cross protection against *Leishmania donovani* infection in Indian langur (*Presbytis entellus*). Taking lead from these results Alum precipitated ALM was evaluated in combination with BCG in this model. Initially a safety evaluation with different doses of Alum-ALM, BCG & Alum-ALM+BCG was conducted. Three monkeys each were separately vaccinated with varying doses of Alum-ALM, BCG (1.0 mg,0.5 mg, 0.1 mg, 0.01 mg) and Alum-ALM+BCG (1.0+1.0 mg, 0.5 mg, 0.1 mg, 0.01 mg, 0.01+0.01 mg) respectively. The monkey vaccinated with Alum-ALM either alone or with BCG were noted to have severe indurations initially till day 10 post vaccination at all the four doses which later developed into bleeding nodules with ulcerations by day 45 post vaccination. Except this no other side reactions such as fever were noticed. In the next experiment 7 out of 10 langurs were vaccinated with Alum-ALM+BCG at the dose level of 1 mg+1 mg/animal and 3 were kept as unvaccinated control. The vaccinated langurs were again observed for post vaccination reactions (induration, erythema, etc.) if any till day 45. The observations were similar as mentioned above. All the monkeys were skin tested on day 60 with ALD, tuberculin and

leishmanin. After 72 hrs post sensitization all the vaccinated monkeys developed positive DTH response (induration) to ALD, 5 to tuberculin and only one to leishmanin. Two out of three unvaccinated control monkeys developed response to tuberculin. All the vaccinated and unvaccinated control monkeys were challenged with 1 x 10^8 amastigotes (i.v). Splenic biopsies of all the langurs were carried out on day 45 p.c. In vaccinated group 2-14 amastigotes / 1000 cell nuclei were observed whereas, in unvaccinated control monkeys the parasite burden was 10-80 amastigotes / 1000 cell nuclei. By day 90 and 180 P.C. in vaccinated group the infection gradually resolved but in unvaccinated control group there was progressive infection. All the animals died by day 118 to 120 p.c.

The findings suggest that though Alum-ALM+BCG vaccine is reactive but is highly protective. Further dose titration and efficacy evaluation of this combination is needed to optimize its efficacy at a safer dose level.

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PROPHYLACTIC EFFICACY OF ALD+BCG AGAINST *LEISHMANIA DONOVANI* IN INDIAN LANGURS.

J.K. Srivastava, Bindu Srivastava, Preeti Sharma, Alka Misra, Anuradha Dube, Sita Naik* and J.C. Katiyar.

Division of Parasitology, Central Drug Research Institute, Lucknow 226 001

** Department of Immunology, SGPGIMS, Lucknow*

Cross protection of autoclaved *Leishmania major* (ALM) (a WHO vaccine developed for cutaneous Leishmaniasis) has been established against V.L. in Indian langurs. Encouraged with the results, autoclaved *Leishmania donovani* (ALD) was employed to assess its prophylactic potential in monkeys against *L. donovani* challenge. A group of langurs of 3.5 - 4.5 kg body weight was intradermally immunized with ALD + BCG (1 mg each) with two booster doses given at interval of 21 days. Similarly, ALM + BCG combination, was also given to another group for comparision. A third group was kept as unvaccinated control. On day 50 of the last dose all the langurs were tested for DTH response using ALM, ALD and tuberculin as sensitins (0.1 mg each). After 10 days, all the langurs were challenged intravenously with 100 million amastigotes isolated from the spleens of infected hamsters.

For enumerating the prophylactic efficacy, splenic biopsys was carried out on day 40 post challenge. Animals immunized with ALD + BCG registered 87-96% parasitic inhibition in comparision to unvaccinated controls. ALM + BCG vaccinated group too recorded similar parasitic inhibition to the tune of 88-98%.

Thus, ALD + BCG can be further exploited as a potential vaccine against V.L. and warrants clinical evaluation.

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SHRI DHANVANTARI --- IMMUNOTHERAPY IN AYURVEDA

क्षमता

VAIDYA : M.B. KULKARNI
Ashwinikumar Ayurveda Chikitsalaya, Ron, 582 209 Dist : Gadag, Karnataka

In our Ayurveda has given importance to Immunity क्षमता and then line of treatment. Because-

ई खल्वायुर्वेदप्रयोजनं - व्याध्युपसृष्टानां व्याधि परिमोक्षः

स्वस्थ्यस्य स्वस्थ्यरक्षणं ज ॥ सु.सू.।

Ayurveda has got two properties :-

1. Curing of the diseases
2. Protection for maintaining of sound health.

Particularly Ayurveda has given plenty of guidelines for maintains of sound health. This is the only one Science regarding this subject viz - Daily routine life दिनचर्य

Seasonal life ऋतुचर्य

Conducts of Morals सद्वृत

If we follow this method, automatically resistance power will increase and person has got natural immunity. so,

व्याधिक्षमत्वं व्याधिपलविरोधितं व्याध्युतप्रतिबन्धकत्वमिति यावत । चक्रपाणि

By this quotation, we came to know the purpose of Ayurvedic system that has given preference to build immunity by way of Indian Medicine,. In Ayurveda, immunity is classified into 3 types.

1. सहज (Strength by birth)
2. कालज (Seasonal strength)
3. युक्तिकृत (Artificial Strength)

First two are natural ones. But now we are thinking of third i.e., Artificial strength. Now a days youngsters health has gone down like anything, due to drugs inhabitant activities etc. This is a very very burning problem in developing countries.

Ayurveda has got the answer

भेषजं द्विविधं च तत ।

स्वस्थस्योर्जस्करं किंचित किंचिदार्तस्य रोगनुत । ज.चि. 1/4

Two types of medicines :-

1. Maintaining of good health

2. Curing for the diseases

स्वस्थस्योर्जस्करं यद् तद् वृष्युम तत्रसायनम् । च.चि. 1/5

Though medicine helps for maintaining of good health we call it as rejuvate of RASAYAN

The characteristics of Rasayan in as follows :-

दीर्घमायुः स्मृतिं मेधामारोग्यं तरुणं वयः ।

प्रभावर्ण स्वरोदार्य देहेन्द्रियबलं परम् ॥ च.चि. 1/7

1. Healthy long live

2. Increases memory power

3. Increases grasping power

4. Sound health

5. Just like young chap

6. Increases complexion

7. Good sound

8. Physically as well as mentally be strong

If we implement RASAYAN method automatically resistance power, immunity will increase and prevention for highly infectious diseases like AIDS, Malaria, Brain fever and other communicable diseases.

There are two types of RASAYAN.

1. Indoor management कुटी प्रावेशिक

2. Outdoor management वातातापीक

Indoor management is very very costly one and will not reach common man also. So, I choose outdoor management i.e., VATATAPIKA.

In our classics number of single drugs and compound drugs are describing for this method. After PANCHAKARMA we are going for developing immunity, by way of administering RASAYAN method.

Drugs are using as follows :

1. *Terminalia chebula* हरितकी
2. *Emblica officinalis* आमलकी
3. *Shilajit*
4. *Tinospara Cardifolia* अमृत
5. *Withnia Somnifera* अश्वगन्ध
6. *Centella asciatica* मण्डूक पर्णि
7. *Semicarpus ancardioum* भल्लातक
8. *Clyceyrizla glabra* जेष्टमधु

According to person's physical and psychological constitution we choice the suitable drug for developing Immunity or strength. In Ayurveda, has described animals meat juice--

यावल्लघुत्वादशनं दद्यान्मांसत्सेन च।

बलं हालं निग्रहाय दोषाणां बलकृत् ॥ च.चि. 3/166

If patient has not felt hungry, then we are going to administer forest animals meat juice like buck हरिण , Goat अज , Goose क्रौंच etc.,

by using these meat juice, patient get strength and naturally fightout nature develop against communicable diseases. So with the help of Ayurveda we can increase immunity power and prevents the victim from infectious diseases.

HEALTH FOR ALL

AYURVEDA FOR HEALTH

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PROTECTION OF SYRIAN GOLDEN HAMSTERS AGAINST VISCERAL LEISHMANIASIS BY IMMUNIZATION WITH PROMASTIGOTE INTEGRAL MEMBRANE PROTEINS INCORPORATED IN LIPOSOMES.

Sangeeta Kulkarni and Vinod Bhakuni
Division of Membrane Biology, Central Drug Research Institute, Lucknow 226 001

The disease leishmaniasis manifests itself from the benign self resolving cutaneous to often fatal visceralized version, depending in part on the infecting parasite species as well as on the host immune status at the time of acquisition of the parasite. This protozoan disease has been on upward trend in the recent years and has been recognised as an opportunistic infection in immunocompromised individuals particularly in the patients infected with Human Immunodeficiency Virus (HIV) has further aggravated the situation. Besides this, several of the strains have become unresponsive to the various antileshmanials which are available. This has increased the urgency for newer more effective antileishmanials or a vaccine.

Several studies on the vaccinating ability of crude as well as defined antigens against cutaneous leishmaniasis have been reported. In contrast to cutaneous leishmaniasis, no significant progress have been made for the identification of antigens that could be used as vaccine candidates for visceral leishmaniasis. The total integral membrane proteins of promastigotes of *Leishmania donovani* were extracted using Triton X-114 phase separation technique. These were partially purified either on the basis of molecular weight using centricon or on their binding efficiency with concanavalin A. The protein fractions thus obtained were evaluated; either alone or in their liposomised form in combination with BCG; for their immunization efficacy against *L. donovani* infection in Syrian golden hamsters. The partially purified IMPs in the free form were found to induce significant levels of protection, maximum of about 40 percent. A slight enhancement to about 53 to 59% was observed for immunization of animals with these proteins incorporated in MLV and in combination with BCG. On the contrary, almost negligible protection was observed when hydrophilic proteins were used as antigens. However, immunization of animals with hydrophilic antigens incorporated in liposomes resulted in significant protection of about 48 percent. These observations demonstrate that a mixture of integral membrane proteins from *L. donovani* promastigotes provide excellent protection against visceral leishmaniasis and thus give a rational for their further exploration as vaccine agents.

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EVALUATION OF NATURAL AND TREATMENT INDUCED IMMUNE RESPONSE IN KALA-AZAR PATIENTS WITH A VIEW TO DESCRIMINATE DRUG RESISTANT CASES

A.K.Bagchi, S.Vimal, M.C.Sharma, A.K.Gupta, V.N.R.Das and S.K.Kar
Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna 800 007

Visceral Leishmaniasis is of significant importance as the disease is highly fetal that displays a wide spectrum of immunological response in mammalian hosts immunological status of host and its responsiveness of *L.donovani* plays an important role in the outcome of different clinical manifestation of the disease.

An evaluation of natural and treatment induced immune response of kala-azar patients at various time point of disease course was made to descriminate drug responsive cases at early phase of treatment study population, comprised 75 subjects which represented confirmed VL cases(n=12); VL treated and responsive, (n=20) PKDL(n=10) along with 20 subjects with other infections; AIDS(n=5), Leprosy(n=5), Tuberculosis(n=5), Hepatitis B(n=5) and 13 endemic healthy and control. Study component constituted demonstration of antileishmanial antibody DAT titre while CMI response was estimated by MIF generation respose of T-Lymphocytes. Sera from confirmed and responsive cases gave a high antibody titre and compared to the category of responder cases with other diseases and healthy controls. Further evaluation reflected that MIF level was depressed in confirmed cases (MIF<20%), which however showed significant rise in resposive cases. MIF generation response of T-Lymphocytes in unreponsive cases did not show much improvement from their pretreatment value. Hence MIF % taken at intake and following treatment along with clinical response may help in determining unresponsive cases.

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CERTAIN ASPECTS OF IMMUNITY TO ARTHROPODS WITH SPECIAL REFERENCE TO IMMUNOLOGICAL CONTROL

Placid E. D'Souza
Department of Parasitology,Veterinary College, University of Agricultural Sciences, Hebbal, Bangalore 560 024

The immune responses to ectoparasitic arthropods which are important pests of livestock and also vectors of many diseases has been reviewed. Different parts of arthropods when exposed to the hosts have different effects on the immune system. Earlier, studies were focussed on the exposed parts of the arthropod such as salivary glands for immunogenic

effects. Breakthrough in immunity and immunological methods has been achieved with the discovery that concealed antigens such as from the gut, peritrophic membrane, thoracic muscles and reproductive organs are highly immunogenic and protective in nature. The emergence of the concept of concealed or novel antigens contributed greatly to the evolution of a vaccine against ticks. Exposed antigens are considered beneficial since elevated responses against them can circumvent the immunosupperssion and subversion of immune responsiveness which often accompanies feeding by blood sucking arthropods. However, exposed antigens lack the key advantage of concealed antigens which can provide very high levels of protective immunity. An understanding of the hosts immune response induced by infestation is essential for development of any anti arthropod vaccine. Arthropod molecules which suppress host immunity should be considered as targets. An anti immunosuppressant vaccine might enhance resistance to blood feeding and pathogen transmission thereby allowing full expression of host immunity. A vaccine directed against only the vector or an antivector component in a vaccine against the pathogens transmitted by an arthropod represents a novel approach to control vector borne diseases. The attempts at immunization against biting flies, myiasis, lice and fleas with emphasis on the development of vaccine against ticks will be highlighted.

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THE CELLULAR IMMUNE RESPONSE IN EXPERIMENTAL INTRA VAGINAL TRICHOMONIASIS INDUCED WITH *TRICHOMONAS VAGINALIS* STRAINS ISOLATED FROM SYMPTOMATIC/ ASYMPTOMATIC PATIENTS

Manjeet Kaur, Indu gupta*, N.K.Ganguly, R.C.Mahajan and Nancy Malla**

*Departments of Parasitology, *Gynaecology & Obstetrics, and **Experimental Medicine & Biotechnology, PGIMER, Chandigarh 160 012*

Human trichomoniasis is one of the most frequent sexually transmitted disease with a world wide distribution. The clinical spectrum of trichomoniasis varies from complete absence of symptoms to marked inflammatory manifestations in females. The exact reasons for varied clinical manifestations are still not clear although the host immune response to parasite seems to be an important factor. In the present study, cellular immune response in mice model infected by intravaginal route with *Trichomonas vaginalis* strains isolated from symptomatic and asymptomatic females has been studied. The pattern of cytokine secretions (IL-2, IL-4, IFN- γ and TNF- α) in infected mice were studied to compare the differences in responses, if any, in mice infected with symptomatic vs asymptomatic strains.

T-lymphocytes isolated from vaginal cervical tissues, 7 days post infection showed

significant proliferative response to *T.vaginalis* crude antigen as well as to non-specific mitogens e.g. LPS & Con-A. The significant increase of IL-2 & IFN- γ immune response was observed in mice infected with *T.vaginalis* strains from asymptomatic subjects in comparison to symptomatic patients. However, the IL-4 and TNF- α showed weak response in both the groups of mice. The results indicate that T-cell dependent mechanisms may be responsible in eliminating the infection.

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NATURAL PROTECTION AMONG *PLASMODIUM BERGHEI* INFECTED MICE AND TRANSFER OF PROTECTION

C. Nagaraj and Meera D. Meundi*

National Malaria Eradication Programme, No.8, Bellary Road, NTI Campus, Bangalore 560 003

** Department of Microbiology, Bangalore Medical College, Bangalore 560 002*

Generally, *P.berghei* infection is fatal to the mice. A small study highlights the role of immune protection in *P.berghei* infected Swiss albino mice. Cell mediated immunity which is responsible for this protection could be transferred from naturally protected mice to other mice through transfer of mononuclear white cells of protected mice. No transfer of protection from lactating mother to siblings were observed. Study highlights the mechanism of protection among mice to *P. berghei* infections and shows the limitations in plasmodial vaccine development. Study shows the possibility of pretection of mice against *P.berghei* infection with the transfer of mononuclear blood cells from a protected mice, a new method of immunisation.

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EASY AND COST-EFFECTIVE REPLACEMENT OF FETAL CALF SERUM WITH HUMAN URINE AS AN *IN VITRO* GROWTH SUPPLEMENT FOR *LEISHMANIA DONOVANI*.

Sarman Singh, D.P.Mohapatra, R.Sivakumar and Jugesh Kumar

Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi 110 029

Leishmania donovani is a kinetoplastid protozoan parasite which causes visceral leishmaniasis or kala-azar. The disease claims several thousand lives every year in Indian sub-continent. The parasite can be grown in cell free media supplemented with fetal calf serum (FCS). Urine from human beings and other mammalian species has also been reported stimulating the growth of *Leishmania* species. However, growth stimulatory effect of urine

has not been studied so far on *Leishmania donovani*. Therefore, we wanted to study the feasibility of culturing *Leishmania donovani* promastigotes in M199 tissue culture medium supplemented with 10% human urine and compared the growth kinetics and antigenic and genotypic characters with cultures obtained by supplementing M199 with 10% FCS and 5% human urine plus 5% FCS separately. Growth was monitored by promastigote counts at different time intervals. SDS-PAGE and Western blotting of the total soluble antigens and kDNA RFLP was carried out to know whether any change occurs in the antigenic and genotypic level of the parasite. The growth curve showed no significant difference in the promastigote counts in all culture groups. Amongst the urine samples from different volunteers, best growth was observed in the culture supplemented with the urine of post-menopausal women. No difference in the patterns of antigenic bands and RFLP pattern was seen. These parameters showed that no alteration occurred in strain specific characters of parasites cultured under three conditions. Urine is a waste product and if it can replace the animal blood product in all tissue culture media used for various parasites, India will save several million dollars on import of FCS. It will also avoid animal sacrifices.

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EXPRESSION OF REPORTER GENES IN CELL LINE AND LARVAE OF MOSQUITOES

Prafulla K.Chandra and Dileep N.Deobagkar
Molecular Biology Research Laboratory, Department of Zoology, University of Pune, Ganeshkhind, Pune 411 007

The best way to understand the molecular basis of mosquito -parasite specificity and interactions is to generate mutations or deletions in those genes in the mosquito genome which are involved in such interactions. This can be achieved by developing vectors and protocols for introducing external DNA elements transgenically. Very few reports of partial success have appeared recently using selected species of mosquitoes. However, none of them pertain to *Anopheles stephensi* - the major urban malarial vector in India. We have developed techniques to introduce and express the derived plasmid vectors with reporter genes in *in-vitro* cultured cells of *A. stephensi* as well as in the larvae of *Aedes aegypti*. In both cases, by using different vectors, β-galactosidase and Green Fluorescent Protein (GFP) could be expressed. Fragments of middle repetitive DNA from *A. stephensi* (designated as Asm) have been cloned and sequenced. One of the Asm 16 was co-transfected with the above plasmids in order to generate stable transformation by integration of introduced DNA in the chromosomes. The generation of stable transformation has been analysed by monitoring the expression of reporter genes as well as localisation of inserted DNA on metaphase chromosomes. A heat shock promoter from *A. stephensi* has been isolated and sequenced, which will be used to develop a homologous promoter based transfection vector for *A. stephensi*.

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CHARACTERIZATION OF *GIARDIA LAMBLIA* GROUP A AND B ISOLATES BY RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS

Ajaib Singh Paintlia, Ramesh Chander Mahajan*, Anuradha Chakraborti and Nirmal Kumar Ganguly

*Department of Experimental Medicine & Biotechnology and * Department of Parasitology, PGIMER, Chandigarh 160 012*

Giardia lamblia (Syn. *G.doudenalis; G.intestinalis*) infection in young adults leads to acute/chronic diarrhoea in some individuals and asymptomatic in others. Isoenzyme and molecular biological studies have separated *G.lamblia* strains into group A also called 'Polish' (symptomatic) and group B also called 'Belgian' (asymptomatic). In the present study, ten *G.lamblia* isolates obtained from symptomatic (five cases) and asymptomatic (five cases) persons were characterized by random amplified polymorphic DNA (RAPD) analysis. This showed homogeneity for eight primers except two primers A_{02} and B_{05}, which separated group A isolates into two rapdemes A_{R1} and A_{R2} and group B isolates into four rapdemes B_{R1}, B_{R2}, B_{R3}, and B_{R4}. Further phenetic analysis showed average genetic distance of 0.105 within group A and 0.121 within group B *G.lamblia* isolates on Jaccord's distance scale, which suggest that both lineages appears to be consists of range of variants with no significant ($p < 0.05$) genetic diversity. The results were compared with isoenzyme analysis which showed positive association and indicated that RAPD analysis could be a useful substitute to isoenzyme analysis.

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MOLECULAR CLONING OF PSA-2 GENE OF *LEISHMANIA DONOVANI*

Nidhi Gupta, Anil K. Rastogi* and Sita Naik

Department of Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226 014

** Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001*

Members of the genus *Leishmania* are responsible for a wide spectrum of diseases varying from the fatal visceral form to the self-healing cutaneous lesions. The causative agent of visceral leishmaniasis (kala-azar) is *Leishmania donovani*, that exists as the flagellated, elongated promastigote in the vector sandfly, and as the intracellular, nonmotile amastigote within the mammalian macrophages. The prevalent antileishmanial drugs are few, cause severe toxic side-effects and the regimen used is lengthy and not always successful. The situation is further compounded by the emergence of resistance in several parasite strains. Vaccines are

more cost-effective, easy to administer and so offer an alternative approach. The present study was done with an objective to clone and characterize the gene encoding *PSA-2 protein* of *L.donovani*, as a possible vaccine candidate. PSA-2 belongs to GPI-anchored family of glycoproteins expressed in most of the *Leishmania* species. The expressed PSA-2 of *L.major* has shown considerable protection in a murine model. We extracted total genomic DNA of *L.donovani* (strain *Dd8*, causative agent of Indian kala-azar), and designed primers based on conserved PSA-2 sequences from other *Leishmania* species. DNA was amplified by PCR using these synthesized primers, Genomic DNA was digested with several restriction enzymes, and Southern blotting was done by DIG-labelling the amplified PCR product as probe. Restriction mapping and Southern analyses reveal the presence of PSA-2 coding gene in this pathogenic, viscerotropic strain of *Leishmania*. The results of all these experiments will be discussed in detail.

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MOLECULAR AND IMMUNOLOGICAL CHARACTERISATION OF A *BRUGIA MALAYI* L3 STAGE SPECIFIC GENE, ALT-1, PUTATIVE VACCINE CANDIDATE FOR HUMAN LYMPHATIC FILARIASIS

R Sabarinathan*+, R.B. Narayanan*, J.S. McCarthy+, K Jayaraman* and P. Kaliraj*

** Centre for Biotechnology, Anna University, Chennai 600 025*

+ Dept. of Medicine, University of Western Australia, Fremantle Hospital, Fremantle, WA 6160, AUSTRALIA

Lymphatic filariasis caused by the filarial nematode parasite *Brugia malayi* and *Wuchereria bancrofti* puts at risk more than a billion people world wide. Genes from the infective L3 stage of the parasite have been identified as potential candidates for vaccine development. One such gene ALT-1, the Abundant Larval transcript -1, is a promising one. PCRanalysis of the various stage specific cDNA libraries using insent specific primers revealed that the ALT-1 was expressed only during the L3 stage of the parasite life cycle. The *B. malayi* ALT-1 gene is 660bp in length with an ORF of 336bp, coding for a 12.7kDa protein. BLASTN/X analysis of the sequenced *B. malayi* ALT-1 gene shows 80% homology to the *D. immitis* 20/22kDa protein, a vaccine candidate in dog heart worm disease. The gene was subcloned in T7 prokaryotic expression system (pRSET) and expressed by induction with IPTG. The expressed protein was purified by IMAC using FPLC. The antigenic nature of the protein was assessed by measuring the humoral response in an endemic population. 0/25 (0%) of Non Endemic Normals, 18/25 (72%) of Endemic Normals, 9/25 (36%) of microfilaremics and 14/25 (52%) of chronic patients in an endemic area showed reactivity with the protein. The high reactivity in the endemic normals indicates that the protein might be involved in the protective immunity, hence was selected as a vaccine candidate. The ALT-

1 DNA vaccine constructs were generated in Vr1020 plasmid. The immunogenic potential of the DNA vaccine constructs and the recombinant protein were analyzed by immunizing CBA mice. The humoral and cellular immune response of the DNA immunized showed a profile similar to that in mice immunized with recombinant protein albeit of a lower magnitude.

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STUDY OF MODULATION OF IMMUNOGENICITY OF PEPTIDES DERIVED FROM THE CS PROTEIN OF *PLASMODIUM VIVAX* USING NOVEL ADJUVANTS.

B.E.Thomas[1], W. Hag[2] and D.N.Rao[1]

1) Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029

2) Department of Biopolymers, Central Drug Research Institute, Lucknow 226 001

The circumsporozoite (CS) protein present on the surface of sporozoites is the most well characterized among the several malarial antigens being considered for developing an effective vaccine. The central repeat region of this protein has been tested both in sub-human primates and humans, but the clinical trials have showed disappointing results. Multiple factors like lack of efficient T-cell help, parasite polymorphism, lack of permissible adjuvants and effective mode of delivery have been contemplated for this inconsistent results. In this study we focused our attention on the central repeat region of *Plasmodium vivax* (both type 1 and 2) as well as region II, a putative hepatocyte binding domain which was extended to include a dominant T-cell epitope. Region II, present towards the C-terminus of CS protein is highly conserved in all malarial parasites. This domain is also conserved in few of the biologically significant proteins like thrombospondin and properdin, which makes it difficult to raise antibodies against this region. For this study, five immunomodulatory adjuvants - three analogs of a human ß-casein fragment and the two glycopeptides of muramyl dipeptide (MDP) were tested with one of the repeat region peptide (GDRAAGQPAGDRAAGQPA). Different haplotype of inbred mice were immunized with peptide antigen in either alum or entrapped in liposomes along with/without adjuvants. Entrapment of peptide in liposomes reduced the dose three folds as compared to alum delivery. Out of the five adjuvants tested, one of the casein fragment and the two MDP derivatives showed maximum modulation of the immune response. This was followed by co-immunization of one of the casein fragment analogs and a MDP glycopeptide with the region II plus T-cell sequence (EWTPCSVTCGVGVRVRSRVNAAN). The highest immune response was observed with the inclusion of casein fragment analog for both alum and liposomal delivery as measured by peptide specific antibody levels and end point titres. Also, the inclusion of adjuvants resulted in alum delivered antigens generating an immune response similar to liposomal encapsulated delivery of antigens. So, the addition of a novel adjuvant resulted in enhancement of immune

response to a sequence which due to its role in hepatocyte adhesion will prove crucial in the development of an effective malaria vaccine.

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METHEMOGLOBIN TOXICITY OF 8-AMINOQUINOLINES AND RELATED COMPOUNDS

Pratima Srivastava, S. Singh*, G.K.Jain*, S.K.Puri and V.C.Pandey**
Divisions of Biochemistry, Pharmaceutics and Microbiology**; Central Drug Research Institute, Lucknow 226 001*

Methemoglobin, a toxic ferric form of hemoglobin is continously formed in the normal erythrocytes, but during abnormal situations *in situ*, the level of the same is enhanced, 8-amino-quinolines and related compounds are causative agents for methemoglobin formation. Employing oxyhemoglobin, the methemoglobin toxicity was about 6 times more with primaquine as compared to CDRI compound 80/53 at 10-9M concentration. Methemoglobin reductase activity was also completely inhibited by Primaquine whereas, 24% inhibition was noticed in case of 80/53 at the same concentrations. Mastomys, rodent animal model was found to be equally good for comparative evaluation of methemoglobin toxicity. Further, the use of primaquine transdermal tape of Mastomys depicted the rise in methemoglobin with increase in length of time. In conclusion, the study presents an *in vitro* simple, economical and less time consuming methods for evaluation of methemoglobin toxicity and also, *in vivo* without employing conventional beagle dog model.

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MICROSPORIDIASIS : THE CURRENT STATUS

T.Anitha Sironmani
School of Biotechnology, Madurai Kamaraj University, Madurai 625 021

The microsporidia were first recognized in last century when *Nosema bombycis* was found to cause the destructive pebrine disease in the commercially important silkworm *Bombyx mori*. Since then, they have been shown to parasitize many other eukaryotes like fish, bees and locusts. In 1985, however, *Enterocytozoon bieneusi* was discovered to cause chronic diarrhoea in HIV- infected patients and subsequently, the number of microsporidian pathogens described in human has grown considerably. Here silkworm is used as a model system for microsporidian characterization. A Western blot method has been developed to identify the microsporidian infection in *B. mori*. An immunodominant 17 kDa protein was indentified in infected samples. This polpeptide seems to be processed from a high molecular weight Zymogen. DNA Finger Printing was used to characterize the different species of *Nosema* that infects silkworm. In order to characterize the genome of *Nosema*, techniques

like Karyotyping, Pulsed Field Gel Electrophoresis (PFGE) and Polymerase Chain Reaction were applied. *Nosema* DNA moved as 23.0 kb and in some cases as 15kb fragment on standard agarose gels and the karyotype showed 4 chromosomes. The PFGE analysis also showed two bands of size 1.35 Mb and 0.97 Mb and each band representing two chromosomes. In some cases either the 1.35 Mb or 0.97 Mb bands were observed. Arbitrarily primed PCR with various primers gave amplification products of size ranging from 1.6kb to 0.15kb. PCR with specific primer showed an amplification product of approximately 350 nucleotides. This can be used as DNA probe for diagnostic purpose. The PFGE, Western blot and bioassay of different isolates of *Nosema* suggest that the same strain behaves differently during different stages of life cycle.

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ARTHROPOD PARASITES ON WILD AVIFAUNA IN THE TROPICS : SOME ECOLOGICAL CONSIDERATIONS

A.K.Chakravarthy and K.Srihari
Department of Zoology, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065

Arthropods mostly affect as ectoparasites living on skin or feathers of birds. Parasitism occurs when birds nest, roost, flock or feed in mixed species flocks. That is aggregation of birds favour infestation by arthropods like lice, of species *Menopon, Menacanthus, Lipeurus; fleas, Echidnophaga,* mosquitoes, *Aedes*; midges, *Culicoides*; flies, *Musca* and mites, *Ornithonyssus* and *Knemidocoptes*.

The problem of arthropod parasitism is more severe in tropical countries due to the diversifed niches and habitats for birds they offer. Bird parasites posing zoonotic problems are documented. The occurrence of Kyasnoor forest disease in parts of Shimoga, Karnataka is transmitted through haemaphysalid ticks believed to be borne on birds. Such cases in the tropics are reviewed and details are discussed in the paper. But pest outbreaks on wild avifauna are not well documented because, surveillance and monitoring systems for arthropod parasites are poorly developed and executed in the tropics.

Literature review revealed that groups of birds such as ducks, geese, pigeons, sparrows, turkeys, Quails, pheasants and other selected passerines are the ones most often vulnerable to infection by arthropod parasites. Wild birds may pickup ectoparasites from mammals during the course of mutualistic interactions particularly during foraging. For instance, egrets, *Egretta garzetta* with cattle and drongos, *Dicrurus adsimilis* with bison, *Bibos gaurus*. Co-evolutionary changes in the host may bring about adaptive changes in parasites. That bird migration effect long distance distribution of arthropod parasites is evidenced with examples.

Many wild bird species suffer from the same parasites as that of their domestic counterparts. Parasites like ticks and mites are host-specific and spend their entire life cycle on the host-bird, while mosquitoes, bedbugs and fleas wander from bird to bird. Proper hygiene, good management, wild-bird proof housing, quarantive protocols, coordination among countries and regular parasitic disease surveillance and monitoring may prevent outbreak-situations due to arthropod parasites in the tropics.

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STUDIES ON HEALTH PROMOTING ASPECTS OF BIFIDUS FERMENTED MILK BY THEIR INHIBITORY ACTION ON ENTERIC PATHOGENS

P. Gajalakshmi, M. Triveni, and A.M. Natarajan
Department of Microbiology, Maharani's Science College, Bangalore and R & D Laboratory of K.C.Das Pvt. Ltd., Bangalore

Bacillus bifidus communis was first discovered by Henry Tissier in the faces of infants. The exact source of *Bifidobacteria* gaining entry into the intestinal tract of nursing infants is not yet known precisely. A stable microflora develops in the colon and faces within three to four days of birth consists of high percentage of Bifidus found nowhere else in nature. It is very difficult for *in vitro* isolation and cultivation of anaerobic Bifidus organisms. Human milk contains Bifidus factor which is responsible for growth and dominance of this *Bifidobacterium bifidum* in infants. Rich population of this species is necessary for their colonisation in the gastro intestinal tract which would ensure building up of normal balance of the intestinal flora by eliminating undesirable types. Therefore, two aspects were taken up in this investigation by using anaerobic strains of *Bifidobacterium longum*. Our studies revealed that milk supplemented with *B. longum* culture containing Bifidus factors such as lactulose and N-acetyl glucosamine gave a rich harvest of Bifidus organisms, i.e., 10"/gm of fermented milk. The filterate of this fermented milk showed inhibitory zones against the seed culture of all the three selected enteropathogens, viz., *Staphylococcus aureus, Escherichia coli* and enterotoxigenic strains of *Bacillus cereus*. The inhibitory zone measured are 29mm for *S.aureus*, 35mm for E.coli and 37 mm for *B.cereus*, in the case of *B.longum* fermented milk filterate. The results obtained on *in vitro* studies revealed the potential advantage of Bifidus fermented milk in controlling enteropathogens and this contribute to emerging up of a new product endowed with health giving properties.

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GENETIC STUDIES OF "GREYISH BROWN" LARVA IN THE MALARIA VECTOR *ANOPHELES STEPHENSI* LISTON

A. Dinesh Madhyastha and N.J. Shetty
Centre for Applied Genetics, Bangalore University, Bangalore 560 056

Genetic studies of mosquitoes, especially of species and strains which are vectors continue to be an essential component of genetic control strategies aimed at disrupting the transmission of diseases. These studies are required to verify the taxonomic status of the geographical isolates of *Anopheles stephensi* and for the design of strains with high genetic variability needed for control of this vector.

The present paper describes the isolation, establishment and genetic studies of a spontaneously occurring larval colour mutant, greyish brown (grb) in *An. stephensi*. The mutant isolated from the laboratory maintained strain of *An. stephensi* which originally collected from Poona. The greyish brown colour appears in the late I instar larva and becomes very conspicuous in the late larval instars. The colour also persists in the pupal stage and the freshly emerged adults. Reciprocal crosses were made between the mutant and wild type. Part of the F_1 individuals were inbred to get F_2 generation and the remaining mosquitoes were back crossed to parental type. The data on the mechanism of inheritance of greyish brown clearly showed that the gene *'grb'* is an autosomal and recessive with full penetrance and uniform expression in both sexes. The viability of mutant is excellent. Therefore, the mutant gene *'grb'* is an excellent marker for *An. stepehnsi*.

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DEVELOPMENTAL EXPRESSION OF DOUBLE STRANDED DNA BINDING PROTEINS IN MALARIA VECTOR, *ANOPHELES STEPHENSI* (CULICIDAE : DIPTERA)

Sunita Singh*, S.K.Gakhar and Harish Shandilya
Biosciences Department, Maharshi Dayanand University, Rohtak 124 001

** Entomology Research Unit, School of Studies in Zoology, Jiwaji University, Gwalior 474 011*

The qualitative and quantitative analysis of double stranded DNA- binding proteins (dsDBPs) have been studied by DNA - cellulose chromatography, SDS-PAGE, spectrophotometry and densitometric scanning during the development of *Anopheles stephensi*. In total, 58 dsDBPs were identified during various stages of development. Two high molecular weight dsDBPs - 98 kDa during all the stages of post -embryonic development and 190 kDa polypeptide during pupation and adult life were common. Various stage-specific dsDBPs

have been identified. Two dsDBPs - 24 & 56 kDa were embryo-specific. Two other dsDBPs -67 & 77 kDa were present exclusively in larva. The expression of 4 dsDBPs -84, 102, 155 & 187 kDa - were observed to be female-specific. Various DBPs identified during the development have been characterized on the basis of their binding strength i.e., on the basis of their elution pattern by increasing salt (NaCl) concentration. Four DBPs (39,43,70 & 79 kDa) have been identified which possess binding affinity only for dsDNA during the development. The changes in the major dsDBPs during development and their probable role will be duscussed in relation to the characteristics analyzed.

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THE POTENTIAL OF ETHNOMEDICINE AND PHYTOCHEMICALS IN THE MANAGEMENT OF PARASITIC DISEASES

C. Kameswara Rao
Department of Botany, Bangalore University, Bangalore 560 056

Mammalian parasites of various plant and animal groups have co-evolved with their hosts. Although the biology of parasites and parisitism was not understood in the past in the same way as it is oday in the light of the advances made in microbiology and parasitilogy, all indigenous systems of medicine have recognised most of the diseases and found largely successful remedial measures. Till the allopathic drugs rooted in synthetic pharmaceutical chemistry took over, plant based medicines were the only recourse to manage parasitic diseases. The earlisest successful drugs against several diseases such as malaria, amoebiosis, intestinal parasites, etc., have all been chemical compounds from plants.

The effectiveness of a large number of synthetic drugs has been rapidly waning off, due to a number of pathogens acquiring resistence to the once very potent drugs. New strains of pathogens, which are no longer susceptible to the drugs have emerged. As a result, diseases like malaria, tuberculosis, leprosy, etc., once considered under control, again pose a serious threat to vast human populations the world over.

During the past three decades or so, a large number of phytochemica compounds has been tested *in vitro* and found to be effective against a majority of plant and animal parasites affecting man. However, their potential has not been adequately recognised.

Basing on representative examples from ethnomedicine, ethnopharmacology and biological activity of phytochemical compounds, the large potential of plants in providing many useful drugs against parasitic diseases will be projected in this presentation.

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A REVIEW OF RESEARCH ON *TRYPANOSOMA EVANSI* IN INDIA

P.D. Juyal
Department of Veterinary Parasitology, College of Veterinary Sciences, Punjab Agricultural University, Ludhiana 141 004

Trypanosoma evansi causes 'Surra' in domestic animals in India. It is a highly pathogenic disease in horses and dogs, while the infection is subclinical in cattle and buffaloes. Since the discovery of *T. evansi* by British Veterinarian Griffith Evans in 1890 in Dera Ismail Khan in erstwhile Punjab, a lot of literature on all accounts of this protozan has been gathered. There is a dire need to develop cheap and proper diagnostic tests. It will also help in outlining the proper epidemiology of the disease in endemic areas. Prolonged use of trypanocides have resulted in the appearance of drug resistant strains in certain areas. These need experimental validation. The control depends upon early diagnosis either by Ag, Ab or by parasite detection. Newer technology help includes DNA probes and PCR based assays for detection of cases of 'Surra' in domestic animals. The paper discusses certain unanswered questions regarding *T. evansi*.

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STUDIES ON INCIDENCE OF ENDOPARASITES OF PIGEONS (*COLUMBA LIVIA*) AND QUAILS (*COTURNIX COTURNIX*) IN AND AROUND TIRUPATI, ANDHRA PRADESH

R.Srinivasa Rao and Md. Hafeez
Department of Parasitology, College of Veterinary Science, A.N.G.Ranga Agricultural University, Tirupati 517 502, A.P.

A study was under taken to record the edoparasites of pigeons and quails, in and around Tirupati, Andhra Pradesh. Out of 162 pigeons examined 61.41 per cent had one or the other infection. Helminths were present in 57 per cent and protozoa were present in 28 per cent of pigeons. The endoparasitic fauna of pigeons recorded was composed of three cestode species, two nematode species and three species of protozoa. The cestodes noted were *Raillietina tetragona, R. echinobothrida* and *R. cesticillus*. Nematodes obeserved were Ascaridia galli and Heterkais gallinarum. The protozoa recorded were *Eimeria species*. *Haemoproteus columbae* and *Trichomonas gallinae*.

Examination of 138 quails (both farm and migratory quails) did not reveal any helminth parasites. But, they were positive for the oocysts of *Eimeria* and *Isospora* species with the percentage incidence of 47 and 42 respectively.

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STUDIES ON THE INCIDENCE OF *TRYPANOSOMA THEILERI* LAVERAN 1902 IN AND AROUND TIRUPATI, ANDHRA PRADESH

N.Ramana Rao, Md. Hafeez and N.S.Charyulu
Department of Parasitology, College of Veterinary Science, A.N.G.Ranga Agrl. University, Tirupati 517 502, A.P.

The present study was taken up to ellucidate the information with regard to the incidence of *Trypanosoma theileri* in white (278) and black (140) cattle in and around Tirupati, Andhra Pradesh. The overall prevalence of *Trypanosoma theileri* in the cattle including white and black cattle was 5.26 per cent. The total incidence in white and black cattle was 1.79 and 12.14 per cent respectively. Both categories of animals put together, the infection rate in adults was 6.05 and in calves below one year old was 1.41 per cent. Prevalence in males and females was 2.01 and 5.69 per cent respectively.

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ISOLATION OF IMMUNOREACTIVE PROTEINS FROM THE LARVAL EXTRACTS OF *BOOPHILUS MICROPLUS* AND *HYALOMMA ANATOLICUM ANATOLICUM* FOR THE DEVELOPMENT OF IMMUNOPROTECTIVE MEASURE

S.Ghosh and M.H.Khan
Entomology Laboratory, Division of Parasitology, Indian Veterinary Research Institute, Izatnagar 243 122, U.P.

Ticks are the most important ectoparasites affecting the livestock industry in the tropical, sub-tropical and temperate regions of the world. The induction of resistance in hosts against ticks seems to be a promising alternative to conventional methods of control which have some well established disadvantages viz., resistance, environmental pollution, residues in milk, meat, hides, skin and natural toxicity.

To develop immunoprotective measure against multi-tick infestation in cattle in India, larval antigens of *Boophilus microplus* (TLEB) and *Hyalomma a. anatolicum* (LSH), vectors for fatal parasitic diseases, were purified by gel filtration and anion-exchange chromatography. Gel filtration (Sephadex G-100) and DEAE-sepharose chromatography of (TLEB) yielded multiple fractions, but only one fraction separated by 0.2M NaCl showed significant precipitation reactions. Similarly, by Sephadex G-200 fractionation followed by anion-exchange chromatography of LSH, an immuno-reactive fraction was isolated. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of reactive fractions revealed the presence of a 68 kDa protein in TLEB and 62 and 29 kDa proteins in LSH are the antigens responsible for

induction of resistance. The study present physiochemical procedures for the purification of tick proteins.

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ANTIFILARIAL EFFECT OF A COMBINATION OF BOTANICAL COMPOUNDS FROM *ANDROGRAPHIS PANICULATA* AND *SANSEVIERIA TRIFASCIATA* ON *DIROFILARIA IMMITIS*

S.C. Datta, P. Sarkar, S.P. Sinha Babu, N.C. Sukul
Department of Zoology, Visva-Bharati University, Santiniketan 731 124, W.B.

Ethanol extracts from the leaves of *Andrographis paniculata* and the rhizomes of *Sansevieria trifasciata* was mixed in the proportion of 1:1 and administered orally on Pariah dogs naturally infected with *Dirofilaria immitis* at dose of 40 mg/kg body weight twice daily for 15 days. The treatment showed a maximum of 46% reduction of microfilarial density on day 15 following the onset of treatment. The microfilarial density started rising after discontinuation of treatment. There was no appreciable variation in microfilarial density in the infected and untreated control animals.

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EXTENSIVE DEMODECTIC MANGE AND ITS THERAPEUTIC APPROACH IN DOG

S. Mittra, V.K. Gupta R.S. Kisthawaria and R.K. Agnihotri
College of Veterinary and Animal Sciences, Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062, H.P.

A non descript hill pup aged about four months was presented to the department of Veterinary Parasitology, HPKV, Palampur. The gross clinical observations were: about ninety five per cent alopacia, wrinkling of skin on the forehead, excessive itching, very weak and emaciated, forelimb were oedematous with pastules and crusts. The animal was biting the lesions continuously to get relief from itching.

The deep skin scraping was taken from the suspected lesions and processed in 10% potassium hydroxide solution. Faecal samples were also examined qualitatively using sugar flotation technique. The microscopic examination of processed skin scraping revealed plenty of *Demodex* sp. in various stages of their development. On faecal examination, it was very interesting to note that the faeces contained very large number of intact *Demodex* sp. In lieu of ova of endoparasites. This could be a sequel of continuous biting of lesions due to itching and parasitic mite could find its orofaecal passage.

The pup (body wt. approx. 5 kg) was medicated with a single intramuscular injection of Ivermectin @ 0.2 ml. Seventy two hours post medication, notable improvement was observed. Itching and biting subsided. Ten days after medication animal looked more alert, cooperative and completely free from scratching and biting. Wooly hairs started appearing on the body surface. Skin scraping did not reveal any stage of *Demodex* sp.

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CLINICAL MANAGEMENT OF SEVERE ANCYLOSTOMIOSIS IN LION

S. Mittra, R.K. Agnihotri and Santosh Kumar
College of Veterinary and Animal Sciences, Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062, H.P.

Literature on parasitism in wildlife is scanty. Cub-hood mortality in lion may pose serious threat in many zoological parks, though sufficient data is not available in this concern. Two lion cubs aged about nine months in captivity from Dhauladhar Nature Park at Gopalpur, H.P. were presented to the faculty clinic of college of Veterinary and Animal Sciences. The presenting complaints were lethargy, swaying gait, severe posterior weakness. The appetite was not reduced. The faeces was mucoid, pasty, pale yellow in colour with moderately offensive in smell. The faecal samples were subjected to qualitative and quantitative examination revealed only strongyle ova resembling of hook worm in heavy concentration for both the samples. Quantitative examination using McMaster technique showed an average 4,900 egg per gram of faeces. The remaining pooled faecal samples (about 15gms) were put into coproculture at 28°C ± 1°C using standard technique. The cubs (body wt. approx.30 kg) were treated orally with Albendazole 400 mg tab(ZENTEL, Smith Kline Beecham) for three consecutive days. The dosage schedule was one tab on the beginning day then two on the second day followed by one tab on the third day. Tablets were embedded inside piece of liver. The larvae were harvested after 96 hours when filariform sheathed larvae were obtained plenty in number. These were identified as larvae of *Ancylostoma* sp. Faecal samples were again examined on seventh day post medication and no parasitic stages could be detected. The condition of the cubs was markedly improved by the time. The epidemiological factors and managemental precautions to be taken to get rid of this situation will be discussed.

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INVESTIGATION ON CROSS ANTIGENICITY AMONGST THREE STRONGYLID NEMATODE PARASITES OF SMALL RUMINANTS

R.L. Sharma and S. Arun Kumar
Division of Parasitology, Indian Veterinary Research Institute, Izatnagar 243 122, U.P.

Studies on cross-antigenicity of three strongylid nematode parasites of major economic

significance viz. *H. contortus, O. columbianum* and *B. trigonocepholum* of sheep and goats was investigated. Two classes of parasite derived antigens, the soluble extract antigen (SEA ; 10, 000 xg supernatant) and the gut integral membrane antigen (GIMA) were prepared and cross-antigenicity amongst the referal nematode parasites was assessed in homologous and heterologous system using species-specific hyperimmune sera raised separately in rabbits. Immunoprecipitation analysis by DID and CIEP demonstraged a close antigenic relationship between *H. contortus* and *B. trigonocephalum*. An immunoperoxidase assay revealed a strong and intense suface reactivity of L_3 of H. contortus in homologous system, in comparison to mild and less surface reactivity in heterologous system. The results of ELISA and inhibition ELISA further substantiated these findings. *H. contortus* showed a greater *B. trigonocephalum* was antigenically closer to *O. columbianum* than *H. contorus*. The SDS-PAGE analysis of SEA and GIMA preparations from the referal nemtode parasites identified 9-13 and 13-14 polypeptides respectively in the molecular range of 22.5 to 97.4 kDa. Purification of gut integral membrane antigen (GIMA) of *H. contortus* was also attempted by Con-A sepharose chromatography.

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cDNA CLONING AND SEQUENCING OF EGG-SHELL PROTEIN GENE OF *FASCIOLA GIGANTICA*

P. Ramasamy And P. Balasubramanian
Department of Zoology, Life Sciences Building, University of Madras, Guindy campus, Chennai 600 025

Egg-shell protein specific mRNA was isolated and purified from vitelline cells of *f. gigantica* by using OLIGO (dT) column. The purified mRNA transcripts were used for PCR amplification. cDNA constructed from the mRNA transcripts revealed seven different bands. They were sub-cloned in bluescript sk +/- vector and one of the clones was sequenced. The DNA sequence data showed a set of 21 nucleotides repeats. The deduced aminoacid sequence of egg-shell protein composition of putative peptide has shown to be predominantly neutral nonpolar aminoacids (62.07%) followed by neutral polar aminoacids (21.11%). The aminoacid composition encoded contains glycine (19.54%), leucine (14.94%), serine (12.07%), proline (11.49%), arginine (6.32%), lysine (5.75%), alanine (5.75%), phenylalanine (4.02%), valine (4.02%), reduced amounts of methionine (0.58%), isoleucine (1.72%). asparagine (1.72%), glutamine (1.15%), histidine (1.15%), aspartic acid (2.23%) and glutamic acid (2.3%). There is a stong correlation between the aminoacid composition of the deduced protein and the chemical composition of the *fasciola* egg-shell. A comparison of cDNA sequences of the egg-shell protein (vitelline cells) of *F. gigantica,* with DNA sequence data bank revealed a significant similarity to high glycine-tyrosine keratin gene of mouse mRNA, *Xenopus laevis* mRNA for xk81b2 keratin exon and *Caenorhabditis elegans* DNA.

These facts plus the mRNA expressed in the vitelline cells of *F. gigantica* suggest that the clone encodes a keratin-like protein.

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MICROTOPOGRAPHY OF EGGS OF FOUR PIGEON LICE (PHTHIRAPTERA, INSECTA)

A.K. Saxena, S.K. Singh, Adesh Kumar, Surman and Smita Badola
Department of Zoology, Government Postgraduate College, Kotdwara (Garhwal) 246 149 U.P.

SEM studies have been performed to record the microtopography of four pigeon lice. Operculum of *Columbicola columbae* and *Companulotes bidentatus compar* exhibits ill defined hexagonal marks which are quite prominent on the operculum of *Hohorstiella lata* (giving bee-hive like appearance). On the other hand, operculum of *Colpocephalum turbinatum* exhibits prominent elongated ridges. Micropyles are found scattered on the operculum of *C. turbinatum* while in other three species they tend to remain lined along the perimeter. Furthermore, egg chorion of upper on fifth portion of *H. lata.* bears spine like apophyses. The egg chorion of other three species does not show any sculpturing/ornamentaion. Egg stigma remains quite prominent in case of *C. columbae*, while in other three species, it is generally obscured inside cementing material used to glue the egg.

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ANTHELMINTIC EFFICACY OF C.D.R.I. COMPOUND 81/470 IN GOATS NATURALLY INFECTED WITH STRONGYLID NEMATODES

R.D. Agarwal and P.P.S Chauhan
Department of Parasitology, College of Veterinary Science and Animal Husbandry, C.S. Azad University of Agriculture & Technology, Mathura campus, Mathura 281 001

Anthelmintic efficacy of C.D.R.I. compound 81/470 (Methy1 (5-(4-(2- pyridiny1)-1-piperaziny1) carbony1) -1H-benzimidazole-2- y1) carbamate) was ascertained in goats naturally infected with strongylid nematodes. For this study, 160 goats between ages 3 months to 8 years and of either sex were selected. All the goats, on faecal examination, were found positive for strongylid nematodes viz., *Haemonchus* sp., *Trichostrongylus* sp. and *Oesophagostomum* sp. E.P.G. (eggs per gram of faeces) of all the goats were estimated which ranged between 700-8000. The goats were divided in to two groups- A (treated) and B(untreated control) with 150 and 10 animals respectively. The goats in group - A were dosed with C.D.R.I. compound 81/470 @ 20 mg/kg body weight orally. The control group of goats were kept untreated. E.P.G. of all the goats were estimated at (-)3 day (prior to dosing) O-day, 7-day, 14-day and 21-day post-treatment. It was observed that on 7 day post-treatment all the

animals became negative for strongylid worm eggs and E.P.G. became zero, while in the control untreated group, E.P.G. of animals remained more or less at the same level as was the case before commencement of study.

Evidently, C.D.R.I. compound 81/470 was cent percent effective @ 20 mg/kg body weight in a single dose given *per os* against strongylid nematode infections in naturally infected goats.

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ANTIGENIC DIVERSITY IN AMPHISTOMES INFECTING *BUBALUS BUBALIS*

S.M.A. Abidi and Wajih A. Nizami
Section of Parasitology,Department of Zoology, Aligarh Muslim University, Aligarh 202 002

Eight different amphistome species, *Gigantocotyle explanatum, Gastrothylax crumenifer, Fishoderius elongatus, Orthocoelium scoliocelium, Paramphistomum epiclitum* (Pink and Yellow varieties), *Calicophoron calicophorum* and *Calicophoron cauliorchis*, were analysed for their antigenic similarity on differences using hyperimmune homologous and heterologous polyclonal anti-amphistome antisera raised in rabbits, by immunoelectrophoresis. Interspecific variations in invoking the antibody response and the maximum number of precipitin arcs were recorded at 8th week of post inoculation. However, different antigenic components with different antigenic potential could be recognised with antisera collected at different intervals which indicates not only antigenic diversity but also antibody turnover. A common precipitin arc, designated as "Arc A" was recognised in all the worms. Variation in antibody titre as determined by ELISA, also revealed intra-specific or strain related antigenic differences particularly in *P. epiclitum* and *Calicophoron* sp. The occurrence of antigenic polymorphism as well as the presence of a common precipitin arc could be significant for the dianosis and immunological control measures of amphistomes.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF *GASTROTHYLAX CRUMENIFER* ANTIGENS AND THEIR POSSIBLE USE FOR IMMUNODIAGNOSIS OF BUFFALO AMPHISTOMIASIS

Gul Ahmad, Mohd. Khalid Saifullah and W.A. Nizami
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202 002

The soluble proteins of *Gastrothylax crumenifer* isolated from the rumen of bufflao

were fractionated on sephadex G-200 column. The column was equilibrated with 0.15M, PBS, pH 7.2. A total of eight major fractions were separated from the whole homogenate of *G.crumenifer*. The nine fractions hereafter referred to as F1, F2, F3, F4, F5, F6, F7, F8 and F9 respectively. Each of these fractions were tested for their antigenicity by ELISA against rabbit hyperimmune sera. The F1 fraction gives maximum antigencity where a dilution of 1:25600 of the test sera could detect this fraction. It was observed that F1, F2, F3 and F4 are highly antigenic while F6 and F7 are moderately antigenic and F5 and F8 are poorly antigenic. The individual fractions when subjected to SDS-PAGE it was observed that F1-F4 are mostly high Mr proteins. F1 has 24, F2, 24, F3, 20 and F4, 12 polypeptides respectively. While F5, F6, F7 and F8 have lower number of polypeptides which are of low molecular weight.

The individual fractions after SDS-PAGE were electrotransferred on immobilon-P protein binding membrane and these transblotted polypeptides were allowed to react with hyperimmune sera. Analysis of results revealed that 2, 5, 3, 4, 3, 2, 3, 3 and 1 polypeptides are antigenic in F1, F2, F3, F4, F5, F6, F7, F8 and F9 fractions of *G. crumenifer* respectively. The maximum antigenic polypeptides were observed in F2 fractions followed by F4. the rest of the fractions were having either 2 or 3 antigenic ploypeptides. The overall results indicates the antigenic polypeptides of *G.crumenifer* are mostly of low molecular weight which ranges from < 14 kDa to 50 kDa. However, antigens of 50, 40, 29, 26, 20, 16 and 15-14 kDa seems to be having some immunodiagnostic potential as these low molecular weight polypeptides are present in most of the fractions. However, further work is in progress to find out the immunodiagnostic applicabilities of these antigens.

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CELL MEDIATED IMMUNE RESPONSE OF DOGS IN *ECHINOCOCCUS GRANULOSUS* INFECTIONS

Gul Ahmad and W.A.Nizami
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202 002

To study the cell mediated immune response (CMI) of dogs in *E. granulosus* infections, ten weeks old dog (*Canis familaris*) puppies were starved 24 hrs. before being given infection. Infection was given orally through gelatin capsule. These animals were tested for delayed hypersensitivity 32 days post infection. It was observed that all the dogs developed delayed type skin hypersensitivity reactions. A positive skin reaction of a diameter greater then 7.5 mm was characterized by a zone of erytheam and induration. These reactions were found maximum at around 48 hours subsiding thereafter. Moreover, the microtitre plate direct assay of killing of protoscoleces of *E.granulosus* provided an excellent system for investigation of

larvicidal events. It was found that immunized cells in the presence of the serum can kill upto 84% of the protoscoleces in 24 hours. After the necropsy of the dogs, the sections of the intestine were looked for the presence of mucosal immune cells. It was observed that there is a massive increase in the number of mast and goblet cells in the intestine of these dogs infected with *E.granulosus*. This indicates that even in the case of *E.granulosus* infection of the definitive host there is involvement of mucosal defense. The exact role of these cells in rejection processes have to be worked out.

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EXTRACELLULAR CULTIVATION AND CHARACTERIZATION OF AXENIC AMASTIGOTES OF *LEISHMANIA DONOVANI*

Nidhi Gupta
Department of Immunology, SGPGIMS, Lucknow 226 014

Members of the dimorphic genus of the parasitic protozoan *Leishmania* are responsible for a wide spectrum of diseases in humans, causing great mortality and morbidity worldwide. The causative organism of visçeral leishmaniasis (*kala-azar*) that is endemic in India is *Leishmania donovani*. It exists as an extracellular, flagellated *promastigote* in the vector sandfly; and as aflagellate, non-motile *amastigote* within the phagolysosomes of mammalian macrophages. This transformation during the parasite's life-cycle involves significant morphological, biochemical and molecular changes. It is the intracellular amastigote stage that is responsible for all clinical manifestations of the disease and is associated with the vertebrate pathology. Hence, vaccines and chemotherapeutic agents need to be developed against this stage. However, studies in this direction have been limited due to the absence of suitable, continuous, axenic culture of amastigotes, that is free of contaminating host cells.

We have developed an *in vitro* culture system for long term generation and maintenance of axenic amastigotes of *L.donovani* (*strain Dd8, causative agent of Indian kala-azar*) in a simple biphasic NNN medium, completely devoid of foetal calf serum (Gupta *et al*, 1996a). These axenic amastigotes were characterized at the morphological and ultrastructural levels (by SEM & TEM), membrane level (microviscosity, lectin-binding studies), biochemically (status of antioxidant and hydrolytic enzyme systems, intracellular metabolities by $[H]^1$-NMR spectroscopy), immunologically (protein-profile, immunoblotting, S35 metabolic labelling) and at the molecular level. The studies revealed a remarkable similarity between the axenically cultured and intracellular amastigotes (Gupta *et al* 1996b). These axenic amastigotes would provide an excellent model for further studies on the molecular biology and immunology of the parasite and could also be utilized for *in vitro* drug screening, development of monoclonal antibodies / vaccines for immunodiagnostic purposes.

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OCCURRENCE OF MANGE MITES IN A COLONY OF SWISS MOUSE AND ITS MANAGEMENT

D.S. Upadhyay, K. Rai, P.Y. Guru and H.B. Rajani
Division of Laboratory Animals, Central Drug Research Institute, Lucknow 226 001, U.P.

Dermatological lesions with mild alopecia, pruritis and reduced consumption of feed were noticed in some animals of laboratory-bred conventional colony of 'Swiss' (*Mus musculus*). The skin lesions were more pronounced on face, neck, shoulder and back. Microscopic examination of skin scrapings of affected animals confirmed the disease condition as 'mange' and revealed *Myobia musculi, Ornithonyssus bacoti,* and *Psorergates simplex* parasites present either singly or as mixed infection. Though, the number of animals affected with these ectoparasites was very low (below 2 percent) as compared to population size of mice available in that colony, the condition was however considered as severe enough to downgrade the productivity as well as the quality of animals. Complete elimination of the disease was achieved by prompt segregation and proper disposal of all affected animals, systemic administration of ivermectin (Glaxo India Ltd., Bombay @200µg/kg, s/c) to the suspected population and use of sterilised racks, cages, bedding and nesting materials at suitable intervals. The factors responsible for entry or transmission of disease, its public health significance alongwith the managemental operations undertaken are discussed in detail.

CONTRIBUTED PAPERS - POSTERS

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MODE OF INHERITANCE OF FENITROTHION RESISTANCE IN *ANOPHELES STEPHENSI* LISTON

Chaitali Ghosh and N.J. Shetty
Centre for Applied Genetics, Bangalore University, Jnanabharathi Campus, Bangalore 560 056

Fenitrothion (O,O - dimethyl - O - nitro - m - tolyl - phospho thionate) belongs to organophosphorous compound group, The present paper describes the mode of resistance in *Anopheles stephensi*. Homozygous fenitrothion (FNr) resistant and susceptible (FNs) stocks were established using WHO diagnostic dose of 0.125 ppm for 24 hour exposure to late third instar larvae. In reciprocal crosses, the F_1 hybrids showed 59.35 and 60.58 % resistance and 40.64 and 39.41 % susceptibility. Results of the back-crosses of F_1 hybrids to parental individuals showed 52.17, 51.64, 51.58 and 51.12 % resistance, while susceptibility was 47.82, 48.35, 48.41 and 48.87 % respectively. Thus the ratio of resistant and susceptible individuals was found to be 1 : 1. The F_2 progeny showed 68.54 and 66.72 % resistance and 31.45 and 33 % susceptibility. The data of genetic crosses between resistant and susceptible individuals revealed that the gene for FNr is incomplete dominant and autosomal as the resistance was shown in both sexes for both F_1 hybrids and back-crosses. The Log-Dosage probit lines for resistant and susceptible, F_1 and F_2 hybrids clearly showed the characteristics imparted by a single incomplete dominant gene. A remarkable variation on the sex ratio, fecundity and egg hatchability was observed between the susceptible and resistant strains.

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SUSCEPTIBILITY STATUS OF *ANOPHELES STEPHENSI* LISTON TO FENITROTHION FROM DIFFERENT GEOGRAPHICAL ZONES IN INDIA

Chaitali ghosh and N.J. Shetty
Centre for Applied Genetics, Jnanabharathi Campus., Bangalore University, Bangalore 560 056

Chemical insecticides continue to be the main component of malaria control in India. The present investigation deals with the comparative susceptibility status of different strains *Anopheles stephensi* to Fenitrothion (O,O - dimethyl - O - nitro - m - tolyl - phospho thionate) - an organophosphorous compound. A total of ten strains of *An. stephensi* were collected from different parts of India - five were from local Bangalore city areas and the remaining each one were from Delhi, Pondicherry, Chennai, Mangalore and Aurangabad. The late third instar larvae reared in the laboratory were used for this study. LC_{50} values in all the strains

varied between 0.0014 and 0.0037 ppm while LC_{90} between 0.0031 to 0.0079 ppm which indicated that *An. stephensi* is highly susceptible to fenitrothion.

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DELTAMETHRIN SUSCEPTIBILITY STATUS IN FIVE SPECIES OF MOSQUITOES AT MYSORE

N. Pushpalatha, J. Urmila, K.N. Ganesh and V.A. Vijayan
Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006

As a part of our investigation on environmental impact of insecticides on mosquitoes of medical importance, larval populations of two Japanese encephalitis vectors, *Cx vishnui* and *Cx. fuscocephala*; two dengue fever vectors, *Ae. aegypti* and *Ae. albopictus;* and a malaria vector, *An. stephensi* were tested for their relative tolerance against a synthetic pyrethroid, deltamethrin. Susceptibility tests were conducted following the standard WHO procedure. The larvae were exposed continuously for 24hr. to different concentrations of deltamethrin and the LC_{50} and LC_{90} values were calculated by probit regression analysis. Thus the LC_{50} values recorded for *Cx. vishnui, Cx. fuscocephala, Ae. aegypti, Ae. albopictus* and *An. stephensi* are 0.00039, 0.000067, 0.00045, 0.00067 and 0.00418 mg/l respectively. This result indicates that *An. stephensi* is the most tolerant among the five as it has 10.72, 62.39, 9.29 and 6.24 times tolerance than *Cx. vishnui, Cx. fuscocephala, Ae. aegypti* and *Ae. albopictus* respectively. Similarly, *Cx. fuscocephala* is the most susceptible in the present test. The observation gains importance as the urban malaria vector is found to be more tolerant and the synthetic pyrethroid tested is now employed in Karnataka for malaria control.

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IMPACT OF PYRETHROID IMPREGNATED BEDNETS ON *PLASMODIUM FALCIPARUM* RE-INFECTION IN A TRIBAL AREA IN ORISSA

T.R.R. Sampath [1], R.S. Yadav [2], S.K. Ghosh [1] and V.P. Sharma [3]
1. Malaria Research Centre, Field Station, E.D. Hospital Campus, Bangalore 560 038
2. Malaria Research Centre, Field Station, Civil Hospital, Nadiad, Gujarat 387 001
3. Malaria Research Centre, 22- Shamnath Marg, Delhi 110 054

A multi village trial of bednets impregnated with Lambdacyhalothrin, Deltamethrin wettable power and Deltamethrin flow were conducted along with untreated bednets and no net control in a malaria endemic area in Orissa during 1990-93. Bednets were impregnated @ 25mg/m^2 at 6 months interval. During this trial *Plasmodium falciparum* re-infection was taken as one of the parameters for assessment of the impact of pyrethroid treated bednets. About 20 % of randomly selected population in each group of villages (pop. 167-376) was

examined for the presence of malaria parasites and the positive cases were given treatment to clear of the parasites. The *P. falciparum* positive cases were treated with sulfadoxine - pyrimethamine for successful clinical cure. All the persons were followed up for one year (September 91 to August 92) with weekly surveillance. Re-infection rate was 32.9 % in the village without nets followed by 15.6 % in the villages with untreated nets. In the three groups of villages with pyrethroid treated bednets the rate was 9.9 to 13.1 %. The relative risk of getting re-infection in villages with untreated nets or with treated nets was significantly lower compared to the village without nets ($P < 0.001$). Compared to the villages with untreated nets the risk in the villages with treated nets was less, but the difference was non-significant. Thus, pyrethroid impregnated bednets seems to be an effective tool in reducing the *P. falciparum* re-infection in highly malarious area.

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OPERATIONAL FEASIBILITY OF INSECTICIDE IMPREGNATED MOSQUITO NETS FOR MALARIA CONTROL IN NORTHEASTERN STATES OF INDIA

Vas Dev
Malaria Research Centre, Sonapur, Assam 782 402

Plasmodium falciparum is the predominant parasite species (>70 %) in the Northeastern States and is responsible for high morbidity and mortality. The Northeastern sector contribute most of the *P. falciparum* cases for rest of India and its proportions are on the increase. To contain the disease, village scale fields trials with "Insecticide impregnated mosquito nets" were conducted as an alternative strategy in Sonapur PHC (Kamrup District) Assam as an intervention measure against *Anopheles minimus* transmitted malaria. Over 2 year (1988-90) study period, more than 70% decline in the malaria incidence were recorded in the intervention area coupled with marked reduction in vector density/man vector contact. Based on the success of these trials, the Government of India launched a pilot project study in all the seven sister States of N.E. region to test the operational feasibility of this method of vector control. One hundred thousand mosquito nets impregnated with deltamethrin (2.5 % flow) were distributed through primary health care centres in malaria ridden pockets of all the seven States beginning 1996. As a follow up study, data on malaria incidence were collected to determine decline, if any, among impregnated net users in the ensuing years. Over 2 years period of compliance in population group of 10,710 in Papum Pare District of Arunachal Pradesh, 92 % decline in malaria episodes were observed over base line incidence. Comparable decline were recorded among impregnated net users in States of Assam and Meghalaya. The much needed public response was overwhelming and further demands are being generated by the respective States to cover additional population groups having greater risk of acquiring malarial infection. These insecticide impregnated nets (popularly known as

medicated nets), have been largely accepted by the communities for personal protection. This strategy is simple, cost-effective, environment friendly and involves community participation, thus holds good promise for malaria / vector control

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A SURVEY ON THE HABIT OF USING MOSQUITO BEDNET AMONG MALARIA PATIENTS OF CALCUTTA

A.K. Mukhopadhyay and S. Mohanti
Regional Office for Health & Family Welfare, Government of India, Hindustan Park, Calcutta 700 029

In Calcutta, specially, *Plasmodium falciparum* cases and death due to malaria show a remarkable increasing trend since 1990. In 1997, area under Calcutta Municipal Corporation (CMC) alone contributed 71.5 % total malaria cases and 51.3 % death due to malaria (Total cases 111032, death 38 in CMC) in comparison to West Bengal (Total cases 155209 and death 74). The malaria expert committee in 1995 identified CMC as "High Risk Area" and identified the metropolise under " Accelerated Urban Malaria Scheme".

Anopheles stephensi is the only vector of malaria in Calcutta. Apart from early detection and prompt treatment and selective vector control measures, Government of West Bengal and CMC has undertaken intensive IEC programmes through mass media to promote personal protection method by use of bednets.

In order to know the habit of using bednet and other personal protection measures, a survey was conducted between August and October 1998, among 836 malaria patients and 342, other people who have not suffered from malaria in the last five years, from three highly malaria endemic wards of CMC.

From the survey, it was revealed that out of 836 malaria patients, 670 (80.1 %) do not use bednets during the sleeping time, whereas 166 i.e., 9.0 % patients have the habit of regular using of bednets in night time. 81.1 % patients were found to sleep in the ground floor, 6.2 % in the first floor. 6.4, 2.4 and 3.9 % found to sleep in the 2nd, 3rd , 4th and above floors respectively during night.

Out of the 166 malaria patients using bednets, 15 (9.0 %) patients were found using defective bednets, 62 (41 %) patients have the habit of going to bed after 23.00 hours in night and 22 (13.2 %) patients shared one bed net along with two or more persons.

Out of those 836 malaria patients surveyed 664 (79.5 %) have no basic knowledge on malaria transmission. 117 (13.9 %) persons were found to adopt other personal protection methods i.e., screening of doors and windows (0.47 %), using mosquito repellent cream and

burning of mosquito repellent coils / cakes etc.,

Of 342 permanent residents of CMC area, who had no malaria for last five years, 331 (96.8 %) were found using bednets regularly during sleeping at night.

From the survey it became very clear that the habit of proper usage of mosquito net has a great value in protection against malaria infection. The study also shows the necessity of giving top priority to the campaign through different mass media in a popular manner in order to increase mass awareness for using mosquito bednets.

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EFFECT OF HIGHER TEMPERATURES ON *ASCOGREGARINA CULICIS* (PROTOZOA, APICOMPLEXA), THE GREGARINE PARASITE OF THE MOSQUITO *AEDES AEGYPTI*

D.T Mourya, C.B. Lande, P.V. Barde, V.S. Padbidri and M.D. Gokhale
National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001

Oocysts of the *Ascogregarina culicis,* the gregarine parasites of *Aedes aegypti* mosquitoes, were found to resist a temperature of 50^0 C up to 5 min. The infectivity of oocysts was almost 100 % when maintained on filter papers at room temperature for 90 days. However, when *Ae. aegypti* larvae infected with trophozoite stages of the parasite were exposed to varying higher temperatures for 60 min, it was found that exposure of 41^0 C to the late second instar not only minimised the host mortality due to heat shock but also eliminated the parasite infection. This technique could be employed to obtain gregarine parasite free colonies needed for carrying out susceptibility studies of insecticides and viruses on the mosquitoes.

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EFFECT OF THERMOTHERAPY ON *NOSEMA BOMBYCIS* NAEGLI PARASITISED EGGS OF *BOMBYX MORI L.* IN DISEASE TRANSMISSION TO PROGENY

Sheeba Revi, M.C. Devaiah*. K.P. Chinnaswamy and R. Govindan
Department of Sericulture, University of Agricultural Sciences, GKVK, Bangalore 560 065

**Dean, University of Agricultural Sciences, GKVK, Bangalore 560 065*

Pebrinised eggs of Pure Mysore breed of silkworm *Bombyx mori L.* of 24 h., 36h. and 72h. age were exposed to temperature of 30, 35, 40 and 45^0 C for duration of 1/2h, 1h, and 2h. The hatched out larvae were reared to cocoons and eggs were prepared. The transmission

of *Nosema bombycis* infection to progeny was confirmed by cellular mother moth examination. Temperature levels had significant influence in reducing the disease transmission to offsprings. The disease transmission to the progeny was 100 percent from the eggs exposed at 30 and 35^0 C and 6.68 percent at 40^0 C, while infection was not noticed in the offsprings obtained after treating at 45^0 C. All the durations of treatment resulted in non transmission of disease at 45^0 C, whereas it was 100 per cent transmission at 30 and 35^0 C. Subjecting pebrinised eggs for thermotherapy at 45^0 C resulted in no disease transmission to the offsprings.

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BIOCHEMICAL STUDIES ON PROTEINS AND ENZYMES IN THE DELTAMETHRIN RESISTANT STRAIN OF *AEDES AEGYPTI* - A YELLOW FEVER MOSQUITO

B.P.R. Angel and N.J. Shetty
Centre for Applied Genetics, Bangalore university, Jnanabharathi Campus, Bangalore 560 056

The present paper describes the quantitative and qualitative studies in the protein and esterase isozyme patterns during developmental stages including eggs, larvae, pupae and adult male and females of deltamethrin resistant and susceptible strains of *Ae. aegypti* for the diagnostic dosage of 0.0001 ppm. From the preliminary studies, it is clear that proteins, alpha and beta esterases are higher in the resistant strains. The appearance of additional bands in the resistant strain reflects the increase in the proteins, alpha and beta esterases.

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THE ROLE OF POST KALA-AZAR DERMAL LEISHMANIASIS IN THE TRANSMISSION OF VISCERAL LEISHMANIASIS THROUGH *PHLEBOTOMUS ARGENTIPES*

D.S. Dinesh, N. Verma, V.N.R. Das, A. Palit, K.Kishore, A.K. Gupta, M.C. Sharma and N.Kumar
Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan. Patna 800 007

Visceral leishmaniasis is a major health problem in Bihar. The disease has been continuing since the beginning of the century. So far infected man is only known as reservoir of the disease. In the endemic areas of kala-azar, there are evidences of the cases of Post Kala-azar Dermal Leishmaniasis (PKDL). The proven vector *Phlebotomus argentipes* is also present in the endemic areas. Hence, the study was designed to know the role of PKDL in the transmission of the VL through *P. argentipes*. This ethically approved experiment was

conducted with the patients admitted at indoor ward of the Institute.

The experiment conducted as xenodiagnosis with 10 patients of PKDL. All were having 1-8 years past history of kala-azar, except one. Macular and maculo-nodular lesions were present in 4 and 6 patients respectively. Patients were parasitologically positive in skin snip except one. One patient was also parasitologically positive for LD bodies, without any past history of kala-azar. Newly emerged laboratory bred *P.argentipes* fed on 25 % glucose solution starved for 18-24 hrs exposed to the lesion of PKDL patients in the batches of 10-25 using feeding cup fitted upon the lesion of patient. Prior to the diagnosis written consent of the patient and guardian was obtained. In total, 227 *P. argentipes* were released for feeding on the lesions of PKDL. Out of which 21.1 % had taken blood meal, of which 50 % died before dissection. One *P.argentipes* had shown positive for flagellate infection after 4th day of infected feeding. The dissected materials were inoculated into culture medium after microscopic examination. Due to contamination the culture was not found successful. The infectivity in susceptible animal with the same parasite will prove the role of *P.argentipes* in transmission of the disease in further more experiments.

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BIOCHEMICAL STUDIES OF INSECTICIDE RESISTANCE IN *ANOPHELES STEPHENSI* - A MALARIA VECTOR, TO CYPERMETHRIN

B.L. Priyalakshmi and N.J. Shetty
Centre for Applied Genetics, Bangalore University, Jnanabharathi Campus,Bangalore 560 056

The development of resistance to various types of insecticides including Organo chlorides, Organo phosphates and Carbamates poses a serious threat to the conventional control measures for vectors, especially mosquitoes. Synthetic pyrethroids are the latest group of potential insecticides with low mammalian toxicity and biodegradability. Cypermethrin a synthetic pyrethroid has a high knockdown effect on insects due to its neurotoxic nature.

The LC_{50} values were computed and the concentration of 0.01 ppm of cypermethrin was fixed as the diagnostic dosage, to separate the laboratory produced homozygous resistant and susceptible strains. By repeated selective inbreeding over many generations, Pondicherry strain of *Anopheles stephensi* was raised as the homologous resistant strain for cypermethrin, and a suitable susceptible strain was isolated from Kengeri (Bangalore).

The present investigation deals with the biochemical estimation of proteins (soluble & total), esterases (α & β), Lactate-dehydrogenase, phosphatases (acid & alkaline) and acetylcholine esterase levels in the different developmental stages such as eggs, larvae, pupae and adults (male & female) of both the resistant and susceptible strains. The protein and esterase

isozyme patterns during the different developmental stages of *An. stephensi* of both the resistant and susceptible strains were analysed using poly acrylamide gel electrophoresis. The zymogram and relative mobility showed variation in the number and intensity of protein and esterase bands in the resistant strain as compared to the susceptible strain.

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RESIDUAL EFFICACY OF CYFLUTHRIN IN A DISTRICT OF KARNATAKA STATE, INDIA

K. Ravi Kumar [1], P.K. Srivastava [2], A. Prakash [4], P.K. Shome [1], C.V. Ponnappan [1], Shiv Lal [3]

1) Regional Office for Health and Family Welfare, Bangalore, Karnataka.
2) Regional Office for Health and Family Welfare, Chennai, Tamilnadu.
3) National Malaria Eradication Programme, Delhi.
4) Directorate of Health Services, Karnataka.

Turuvekere Primary Health Centre of Tumkur District was sprayed with cyfluthrin 10 % @ 25 mg/m^2 in the year 1996, for malaria control activity under National Malaria Eradication Program. To study the residual efficacy of this insecticide on sprayed wooden / cement plastered walls, Cone-Bioassay Tests were carried out using *Anopheles culicifacies* as test insect, which is a known vector in these areas. The study revealed that, 100 % mortality was recorded from both wooden and cemented surfaces upto 15th week which declined to 90 % after 16th week and remained constant upto 36th week on wooden surface. On cement plastered surface, the mortality was reduced to 40 % on 17th Week and the mortality remained same upto 36th week.

Following the next round of spray in the locality which was done about 54 weeks after the initial round, weekly bio-assays were conducted on the same sprayed surface. On cement plastered surface, 100 percent mortality was recorded till 17 weeks. By 22nd week, the mortality came to 71 %. It became 47 % by 24th week and 29 % by 29th week. The mortality reached 18 % by 33rd week and remained so by 36th week. On the wooden surface, the mortality remained 100 % till 17th week. The mortality reduced to 83 % by 22nd week. It became 61 % by 24th week and 43 % by 19th week. The mortality reached 29 % by 36th week.

The longer residual efficacy of this pyrethroid insecticide showed it's promising role as residual insecticide in malaria control.

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EVALUATION OF MUTAGENICITY OF INSECTICIDES EMPLOYED TO CONTROL MOSQUITOES CAUSING TROPICAL DISEASE

N.J. Shetty [1] and F.J. Otto [2]

1) Centre for Applied Genetics, Bangalore University, Bangalore 560 056, India.
2) Fachklinik Hornheide, University of Muenster, D - 48157, Muenster, Germany.

Vector control in India and other developing countries depends mainly on insecticides. Since those insecticides may cause harmful effects on human health, evaluation of toxicity and mutagenicity is necessary in order to be able to protect human beings.

A research project was initiated to assess mutagenicity of three extensively used insecticides, namely Malathion, and the synthetic pyrethroids Deltamethrin and Cypermethrin. Three methods were applied to evaluate mutagenic effectiveness : the microscopic scoring of chromosome aberrations, the microscopic counting of micronuclei and the flow cytometric assessment of increased DNA content variation as measured by the coefficient of variation.

Cultures of Chinese hamster cell line M3-1 clone H and of metabolically active human Hep-G2 cells were incubated with various concentrations of the insecticides for 24 hours. 1-Methyl-3-nitro-1-nitrosoguanidine (MNNG) was used as a positive control.

MMNG exhibited a clear concentration dependent effectiveness in both cell systems and in all three assays. The flow cytometric assay was less sensitive than the chromosome analysis and micronucleus test.

Malathion was found to be mutagenic only in the metabolically competent Hep-G2 cells. It was ineffective in the Chinese hamster cells.

Deltamethrin showed mutagenic effectiveness also only in the HepG2-cells. The effect was detectable by the chromosome analysis and micronucleus test not by the flow cytometric determination of the coefficient of variation.

Cypermethrin appeared to exhibit mutagenic effectiveness in the Hep-G2 cells at least in the chromosome aberration assay, whereas the other tests showed no clear effect.

These results indicate that not only Malathion but also the pyrethroids Deltamethrin and Cypermethrin must be considered potentially mutagenic.

Further experiments, particularly *in vivo* tests are required to substantiate these results.

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IVERMECTIN : EFFECT ON SUGAR METABOLISM IN *ACANTHOCHEILONEMA VITEAE*, A RODENT FILARIAL PARASITE

Amita Shukla-Dave, V.M.L. Srivastava* and R.K. Chatterjee
**Divisions of Parasitology and Biochemistry, Central Drug Research Institute, Lucknow 226 001*

Lymphatic filariasis is a major public health problem in several tropical countries including India. Despite serious efforts made worldwide, no suitable control measure against adult worms could yet be developed. Hence, there exists a persistent need for developing an effective and safe macrofilaricide, Ivermectin (Mectizan) which kills microfilariae of a wide range of filarial species is not lethal to adult worms in filarial patients, However, at higher doses this drug has shown to produce adulticidal action against *Acanthocheilonema viteae* and *Brugia malayi* in rodent host Mastomys. At a dose of 200 µg/kg it kills nearly 74 % of adult *A. viteae* parasites. Thus, in an attempt to delineate the mechanism of macrofilaricidal action of Ivermectin against *A. viteae* in *Mastomys coucha*, the drug was administered orally, at a dose of 250 µg / kg x5 days and the worms were removed from host on day 16 of start of therapy for biochemical assays. The drug treated worms showed enhanced rate of glucose uptake but reduced rate of lactate production. The levels of ATP (Adenosine triphoshate) and PEP (phosphoenol pyruvate) were also found much lowered. Activities of all the 7 glycolytic enzymes (hexokinase, phosphoglucoisomerse, aldolase, phosphofructokinase, enolase, lactate dehydrogenase and pyruvate kinase) assayed in these worms were found markedly subdued.

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ROLE OF HEME / DRUG METABOLISM IN ACQUISITION OF RESISTANCE BY MALARIAL PARASITES

Pratima Srivastava, K.K. Kamboj*, S.K. Puri ** and V.C. Pandey
Division of Biochemistry, Parasitology and Microbiology**; Central Drug Research Institute. Lucknow 226 001*

Malaria, a protozoan parasitic disease is primarily responsible for tolling millions of lives annually, the reason being the acquisition of resistance by the malarial parasites against all the commonly used antimalarials. Continued efforts are being attempted to find out the mechanism of resistance by the parasites. Different views have been put forward in relation to acquisition of resistance, but none could be implicated in solving the problem of the same. The present findings add a step in the existing concepts about malaria resistance based upon the study of heme/drug metabolism.

Heme and drug metabolism are the two important pathways, required to maintain the

heme (a toxic biomolecule, formed as a hemoglobin digestion product, by the intraerythrocytic stages of malarial parasites) equilibrium and antimalarial efficacy. Experiments conducted show that resistance strain of *Plasmodium falciparum* possess higher activities of heme (Synthesis-S-aminolevulinic acid synthase and ferrochelatase; degradation-heme oxygenase and biliverdin reductase) and drug (glutathione-S-transferase) metabolising enzymes. There exist a good correlation between the activities of heme oxygenase and glutathione-S-transferase and the resistance titer of the malarial parasites. Molecular characterization and selective inhibition of these enzymes seem to provide a potential means to explore the mechanisms of drug resistance in malaria as well as to curb the menance of the same.

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A STUDY OF MALARIA IN THE INDIAN AIR FORCE

S.K. Krishnan
Dept of Community Medicine, Dr. B.R. Ambedkar Medical Collage, K.G. Halli, Bangalore 560 042

The Indian Air Force (IAF) has a well established health surveillance system and prides itself with one of the most well organised comprehensive health care systems in the country. The IAF personnel serve in different parts of the country at flying bases which are usually located next to the airports (i.e. outskirts of the city). These bases have a very high level of environmental sanitation. However, even under these so called idealistic conditions, incidence and prevalence of malaria has been showing increasing trends.

Data for the past five to six years were analysed and the results are presented in the study. Prevalence of malaria in the IAF varied from 4.89 / 1000 in 1990 to 7.79 / 1000 in 1995. Prevalence was highest in the western region and ranged from 7.2 / 1000 in 1991 to as high as 13.44 / 1000 in 1995, followed by the north eastern region which ranged from 4.9 / 1000 in 1990 to 13.02 / 1000 in 1996. the northern region had a prevalence ranging from 12.6 / 1000 in 1990 to 9.16 / 1000 in 1996.

Breakdown of the malaria cases show that there has been a gradual reversal from the benign vivax type of malaria to the malignant falciparum type of malaria since 1993-94. This trend is a cause for concern since it indicates a failure of the falciparum containment programme component of the National Malaria Eradication Programme (NMEP).

The study also highlights some of the major factors like migration and tribal malaria which require to be tackled if " Roll back Malaria" is to be successful in the country.

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AMOEBICIDAL EFFECT OF EUGENOL

S.P. Sinha Babu, A. Debnath and P. Das *

Department of Zoology, Visva-Bharati University, Santiniketan 731 235, W.B.

** Division of Parasitology, National Institute of Cholera & Enteric Diseases, Beliaghata, Calcutta 700 010*

Eugenol, a 4-alleyl—2 metohoxyphenol extracted from plant *Ocimum sanctum* L. (Lamiaceae) and a component of clove oil, was assessed for its *in vitro* amoebicidal activity against the axenic *Entamoeba histolytica* trophozoites. The drug was tested at four different concentrations i.e., 100 µg/ml, 250 µg/ml, 500 µg/ml and 1mg/ml. Hundred percent mortality was obtained with 1 mg/ml. The minimal inhibitory concentrations for eugenol and metronidazole were 250 and 10 µg/ml, respectively. No apparent toxic effects were observed in eugenol treated rats.

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EFFECT OF SINGLE-DOSE OF DIETHYLCARBAMAZINE CITRATE ON THE MICROFILARAEMIA IN INDIVIDUALS HARBOURING *WUCHERERIA BANCROFTI* INFECTION

A.M. Khan, P. Dutta, S.A. Khan, N.K. Baruah, C.K. Sarma and J. Mahanta

Regional Medical Research Centre, Northeastern Region, (ICMR) Post Box # 105, Dibrugarh 786 001, ASSAM

Effect of diethylcarbamazine citrate at a single dose schedule of 6 mg per kg body weight was evaluated against bancroftian filariasis in a selectively treated tea workers of Assam. Of the 70 individuals, found microfilaraemic following filaria survey during 1995-96, 56 treated individuals (80 %) were successfully followed on one year post treatment. Thirty three individuals become amicrofilaraemic (microfilaria clearance rate 58.9 %). Individuals who remain microfilaraemic, following chemotherapy, had shown significant reduction in microfilaria intensity (89.8 %) as compared to pretreatment values. Sex and age wise differences in microfilaria clearance rate and reduction in microfilaria intensity were insignificant statistically. Subjects showing microfilaraemia after therapy is probably due to survival of few adult female worms or reinfection as the later can not be ruled out in the present study. Over all, diethylcarbamazine citrate at the dose of 6 mg per kg body weight, given annually appears reasonably efficacious against bancroftian filariasis and seems suitable for mass chemotherapy for the control of bancroftian filariasis.

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DIAGNOSIS OF PLASMODIA IN WET MOUNT PREPARATION

C. Nagaraj and Meera D. Meundi*

National Malaria Eradication Programme, No.8, Bellary Road, NTI campus, Bangalore 560 003

* *Department of Microbiology, Bangalore Medical College, Bangalore 560 002*

Wet mount preparation of blood smear examination is an old technique. However, it was found useful in the diagnosis of Plasmodia; especially in differentiating the species. Use of simple vital stain in the wet mount make the diagnosis rapid and more accurate. Like in the diagnosis of *Theileria,* wet mount was found to be simple and useful in differentiating *P.ovale* from *P. vivax.*

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LACTOPHENOL COTTON BLUE (LPCB) WET MOUNT PREPARATION OF STOOL FOR INTESTINAL PARASITES

M.R. Shivaprakash, S.C. Parija and C. Sheeldevi

Department of Microbiology, Jawaharlal Institute of Postgraduate medical Education & Research, Pondicherry 605 006

Lactophenol Cotton Blue (LPCB) has been used for the first time in this laboratory for wet mount preparation of stool to demonstrate intestinal parasites. In this study, we have examined 340 stool specimen by this method. Each specimen was examined microscopically for intestinal parasites by preparing LPCB wet mount and also by preparing saline and iodine wet mounts of stool. Direct microscopic examination of LPCB, saline and iodine wet mount preparation of stool specimens showed trophozoites cysts and ova of various parasites in 169 specimens. Blue coloured cysts, trophozoites and helminthic ova in stool could easily be detected and identified in the LPCB preparation of stool. A large number of fields in each stool smear could be examined easily in LPCB preparation without causing any strain to the eye. The LPCB wet mount is simple, the reagents are inexpensive and are currently available commercially. We therefore recommended the use of LPCB stain along with saline mount for routine microscopic examination of stools in a diagnostic parasitology laboratory.

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USE OF LACTOPHENOL COTTON BLUE (LPCB) IN THE ANAL SCRAPPING FOR THE DIAGNOSIS OF ENTEROBIASIS

S.C. Parija, C. Sheeldevi, M.R. Shivaprakash and N. Biswal*
Department of Microbiology & Paediatrics, Jawaharlal Institute of Postgraduate Medical Education & Research, Pondicherry 605 006*

Recently for the first time, we used the LPCB stain for the wet mount preparation of faeces. Trophozoites, cysts and ova could be recognised and identified with ease in the stool. In this study, we have used LPCB in the anal scrapping collected by Scotch cellophane tape method. A total of 125 children admitted in the ward and attending Paediatric OPD of this hospital were included. A drop of LPCB was put on a clean microscopic glass slide. Then the strip of clear cellophane tape was held between the thumb and forefinger with the sticky side facing outwards. the sticky side was pressed against the skin across the anal opening with even, thorough pressure. The tape was removed and the sticky side was placed against the surface of glass containing drop of LPCB. From the same patient another specimen was also collected by cellophane tape method and was placed on clean glass slide but without any LPCB. In this cellophane tape method using LPCB, the eggs of *Enterobius vermicularis* were stained deep blue and could be easily detected and identified. This was in contrast to colourless and unstrained eggs of *E.vermicularis* seen in cellophane tape method without LPCB. A total of 34 cases were positive for the eggs of *E.vermicularis* by cellophane tape method using LPCB in the anal scrapping collected by Scotch cellophane tape method for the detection and identification of eggs of *E.vermicularis* in children.

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PHARMACOLOGICAL ASPECTS OF *CENTELLA-ASIATICA*

Singh Mamta, Abbas S Sadiq*, N. Singh* and K.C. Pandey**
Department of Microbiology, Central Drug Research Institute, Lucknow 226 001

**Department of Pharmacological & Therapeutics, K.G's Medical College and International Institute of Herbal Medicine, Lucknow 226 001*

***Head, Department of Zoology, Lucknow University, Lucknow 226 001*

The plant *Centella asiatica* belongs to family umbeliferae known as Brahmi/ Mandookparni in Sanskrit/Hindi has been described to be useful in gastrointestinal disturbances like diarrhoea. It has also been described as a nervine tonic and central nervous system (CNS) active medicine. In the present study, the general pharmacological CNS activity and toxicity were evaluated from the lot of this drug which was collected from Sitapur District, U.P. Plant was thoroughly washed, dried and made in roughly crushed powder. This

powder was extracted with 70 % ethyl alcohol. Alcohol was evaporated and residue thus obtained, suspended in normal-saline. This suspension was administered by oral route to animal (albino mice and albino rats).

Under experimental studies, *Centella asiatica* extract (CAE) did not induce any overt effect on general behavioral and muscular coordination by Rota rot test. However, it possessed mild sedation and reduced he activity of mice as judged by photoactometer. Since the drug have been used in gastrointestinal disorder in ancient literature, its effects were not studied earlier on the mobility and peristalsis of intestine *in vivo*. Its effect on intestinal transit was studied in albino rats by charcoal meal test. The drug was found to reduce the intestinal transit was studied in albino rats by charcoal meal test. The drug was found to reduce the intestinal transit in a dose dependent manner conforming its Ayurvedic-concept for its usefulness in diarrhoeal condition. The range of safety ratio (LD_{50}/ED_{50}) of the CAE was quite high proving the innocuous nature of the drug.

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RECENT MALARIOGENIC SITUATION OF AJODHYA HILL AREA OF DISTRICT PURULIA (WEST BENGAL)

S. Mohanti, A.K. Mukhopadhyay and P. Banerajee
Regional Office for Health & Family Welfare, 49/12B, Hindustan Park, Calcutta 700 029

District Purulia of West Bengal is endemic for malaria transmission since 1970. In 1997, a total of 9932 malaria cases and 11 deaths due to malaria was contributed from this District only. The worst malaria affected area of the District is Ajodhya Hills. It is a tribal dominated area (Tribal population is 60 %), situated in the Chottanagpore plateau very close to Singhbum District of Bihar.

In order to assess the magnitude of the disease, a mass blood survey was undertaken in three villages of the Ajodhya Hill area. An entomological evaluation was also undertaken between September to October 1998.

Out of 1129, population of the three villages, mass blood slides of 450 villagers were examined, of which 121 was fever cases and 329 was non fever cases. Of 450 blood slides examined, 119 slides were found positive for malaria parasites, out of which 90 was *Plasmodium falciparum*. Slide positivity rate and *P.falciparum* percentage was calculated as 26.4 and 75.6 % respectively. Out of 329 non fever cases (the persons have no fever for last 15 days), 47 were found positive for *P.falciparum* infection. In 39 cases, only ring stage and in 8 cases both ring and gametocytes of *P.falciparum* were recorded. Asymptomatic *P.falciparum* cases with both rings and gametocytes (Infective stages) was recorded 1.7 % in the community

during study period.

A total of nine Anopheline species, along with two vector species i.e., *Anopheles culicifacies* and *An. fluviatilis* were recorded during study period. Average Man Hour Density of *A. culcifacies* and *A.fluvitilis* recorded from human dwellings were 0.5 and 1.0 respectively. *A.culicifacies* was found resistant to DDT and dieldrin but susceptible to malathion. On the other hand, *A.fluviatilis* was found highly susceptible to all the insecticides.

From the study, it is revealed that being tribal dominated malarious area and presence of asymptomatic cases, Ajodhya Hill area of West Bengal need special attention for malaria control.

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IDENTIFICATION AND PARTIAL CHARACTERISATION OF IMMUODOMINANT ANTIGENS OF *TAXOPLASMA GONDII* AND THEIR POSSIBLE DIAGNOSTIC APPLICATIONS

D.P. Mohapatra, Jugesh Kumar, R. Sivakumar and Sarman Singh
Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi 110 029

Toxoplasma gondii is an intracellular protozoan parasite. It is one of the leading opportunistic infectious agent in immuocompromised patients. With the increase in AIDS cases in India, cases of toxoplasmosis are also increasing at a logarithmic rate. Serology is the most common method of diagnosing toxoplasmosis. The serological diagnosis of the disease is based on the detection of anti-toxoplasma antibodies in the serum. The serological diagnostic kits are imported from the developed countries at a very high cost. So far no such indigenous diagnostic kit is available in India. Therefore, we are purifying the immunodominant antigens of *Toxoplasma gondii* for their possible diagnostic applications. IgG (anti-toxoplasma antibodies) from the pooled patient sera were purified using protein G sepharose affinity column. These purified IgGs were then linked with CNBr activated sepharose to prepare an affinity chromatographic column. The antigens of the parasite were isolated from the sonicated tachyzoites of the parasite using this affinity column. Further, the antigens were purified using gel filtration column. The molecular weight of the purified antigens were determined using SDS-PAGE and identification of the antigens was done using immunoblot analysis of the purified antigens. The identification and characterisation of the immunodominant antigens are under way, which will further give the picture of diagnostic applications of such antigens.

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VARIATIONS IN THE ACID PHOSPHATASE, ALKALINE PHOSPHATASE AND CREATINE ACTIVITIES OF SWISS ALBINO MICE DURING EXPERIMENTAL ANCYLOSTOMIASIS

B.D.J. Satyalatha and V.V. Vardhani
Department of Zoology, Nagarjuna University, Nagarjuna Nagar 522 510, A.P.

Activities of acid phosphatase, alkaline phosphatase and creatine were studied in heart, liver and skeletal muscles of Swiss albino mice during the course of *Ancylostoma caninum* infection. The values of these enzymes differed significantly in gastrointestinal and extraintestinal phase of infection as compared to control group. Similarly, in drug treated+infected animals also, the enzyme values altered significantly during the infection period. In heart and liver, the level of acid phosphatase showed decreasing trend, while the alkaline phosphatase increased only on day 9 of infection in experimental groups. In gastro and extra gastrointestinal phase of infections creatine activity in skeletal muscles showed higher values. Thus, acid phosphatase, alkaline phosphatase and creatine constituting an important component of resistance during experimental ancylostomiasis.

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EFFICACY OF DAT AS DIAGNOSTIC TOOL IN FIELD POPULATION

Raina Singh, S. Vimal, M.C. Sharma, A.K. Gupta and S.K. Kar
Rajendra Memorial Research Institute of Medical science (ICMR), Patna 800 007, Bihar

Visceral leishmaniasis (VL) is a widespread and potentially fetal disease caused by the trypanosomatid protozoon *Leishmania donovani*. The main problem associated with this disease is that it is diagnosed very late. The diagnosis of the disease has continued to rely chiefly on finding the parasites in the splenic or bone marrow aspirates that are obtained by traumatic and sometimes dangerous procedures. Therefore, there is an urgent need for less invasive method. The North-Eastern India being the endemic area and Bihar being the highly endemic area there is a need for less invasive, simple, specific tool for community survey. As such efforts have been made to evaluate the efficacy of DAT as diagnostic tool which is economical and is feasible in field conditions, as this disease is mainly of rural origin. The study was made to screen kala-azar cases based on early symptoms and manifestations; eg- fever pallor, cough & cold, Hepatosplenomegaly. On the basis of recent report on outbreak of kala-azar, a field survey was conducted. A sample sera of 500 sampled population comprised of 120 recently cured, 160 active cases, 165 follow-up cases and 25 PKDL cases as well as 30 healthy subjects (TB, Malaria, AIDS, Hepatisis) were tested with DAT. Antigen

for the test was prepared from trypsin treated, coomassie brilliant blue stained *L. donovani* promastigotes (DD_8) strain. The test was performed as per the protocol of Harith et. al. (1986). The whole test was performed at room temperature. The details will be discussed.

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MALARIA IN MEDICAL STUDENTS: A STUDY OF HEADACHE AND FEVER AS SPECIFIC SYMPTOMS AND THE USE OF CHEMOPROPHYLAXIS AGAINST MALARIA

G. Praveen Bhat, A. Rajeev and M. Praveen Gopinath
Kasturba Medical College, Mangalore 575 001, Karnataka

87 Medical students who were relatively new inhabitants of the malaria endemic city of Mangalore were questioned about the symptoms during their last experience of febrile disease. The presence of headache during this febrile episode was studied in detail with regard to its nature, site, location, diurnal variation and relief upon cessation of fever. Subjective opinions on the severity of headache and fever were also collected. The pattern of headache and fever was compared and collated with regard to the diagnosis of the febrile episode. It was found that headache of sudden onset as a symptom was 3 times commoner in cases of malaria and the headache tended to be bilateral, frontal, continuous, lasted throughout the day and more often not associated with the presence of fever.

Fever of more than 100^0 C was present in 92 % of diagnosed malaria cases. Out of the 87 students, only 53 % had undergone blood examination in the episode of febrile illness. Hence, the potential reasons for undergoing peripheral blood smear examination were also analysed. It was found that the number of previous attacks of malaria and the severity (both subjective and objective) of fever on the decision to undergo a blood examination for malaria. The number of previous malaria attacks in the subjects who had their blood examined was 0.91 (sd 1.36) and among those who did not, it was 0.39 (0.92) and the difference was statistically significant ($P< 0.05$). The study points to the possibility of subjects either with low grade fever or without the previous experience of malaria tending to neglect or avoid blood smear examination which may point to the need of educating individuals in endemic area to get themselves investigated for all grades of severity of fever.

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CLINICO-PARASITOLOGICAL PROFILE OF RECENT UPSURGE OF FALCIPARUM MALARIA WITH ACUTE RENAL FAILURE

K. Ganesan [+], Bijay R. Mirdha, J.C Samantray [*] and O.P. Malhotra [+]
Department of Medicine [+] and Microbiology, All India Institute of Medical Sciences, New Delhi 110 029

Malaria caused by *Plasmodium falciparum* are increasingly recognised in recent years with associated complications and drug resistance. Renal involvement due to falciparum malaria varies widely. Eight hospitalised patients were infected with faciparum malaria in a short span of six months. Diagnosis was made using conventional Romanowsky's stained blood film, Acridine orange (AO) stained blood film and Quantitative buffy coat (QBC) assay. Sequential parasite count was done every day in each case. HRP-II antigen test was also carried out in few cases. All patients had associated acute renal failure (ARE). Their ages ranged from 17 to 32 Yrs. The onset of ARF was usually within first week of appearance of fever. The probable underlying factors leading to ARF were hyperparasitaemia (100%), intravascular haemolysis (38 %), hepatic dysfunction (25 %) and volume depletion (12.5 %). All of them had received either quinine (injectable or oral) or mefloquine. However, no drug resistance was noted, and mortality was 100 % . Thus, the present study implicates that falciparum malaria complicated with acute renal failure warrants vigorous monitoring as well as alternative treatment to prevent inevitable morbidity and mortality.

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RELATIONSHIP BETWEEN MALARIA AND SOCIO-CULTURAL ASPECTS IN KHEDA DISTRICT, GUJARAT

H.C. Srivastava and R.M. Bhatt
Malaria Research Centre, Civil Hospital, Nadiad 387 001, Gujarat

A study was carried out on the relationship between malaria and socio-cultural practices in 12 villages (population 39800) downstream of Wanakbori weir on river Mahi in Kheda District during 1994. The methodology comprised of case detection and collection of information regarding soci-cultural practices. Based on fortnightly surveillance a total of 1781 febrile patients were screened for malaria (SPR-14.8 % and *P.falciparum* - 53.09 %). Socio-cultural practices of 1650 febrile cases among whom 259 had malaria were analysed. There was variable degree of malaria in different groups. A striking relation was noticed between malaria and social groups, profession, sleeping habit and economic status of respondents. Malaria incidence was relatively higher in scheduled castes (SPR-22.3 %), dependents which included children and old persons (16.6 %), farmers (15.4 %) and in low income group

(12.5 %). The people sleeping indoors (73.5 %) and those who did not move out of the village (92.3 %) had lower SPR i.e., 15.1 and 15.9 %, respectively. The treatment seeking behaviour of the people did not show any association with malaria although more than 60 % patients took treatment after three days of onset of fever. Among personal protection devices, use of fan, mosquito nets and other measures were found promising as morbidity (SPR) among users was 8.8, 9 and 9.5 % respectively which was significantly low. These observations indicate significant relationship between malaria morbidity and socio-cultural practices.

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TRIBAL AND FOREST MALARIA IN ORISSA, INDIA

N. Mohapatra[1], R.K. Das[1], D.P. Hansdah[1], B.P. Patnaik[2], R.K. Hazra[1] and S.K. Parida[1]

1. Division of Medical Entomology & Parasitology, Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar 751 016, Orissa

2. Regional Director, Health & Family Welfare, BJ-25, B.J.B. Nagar, Bhubaneswar 751 014, Orissa

Resurgence and increasing incidence of death due to malaria has been creating an alarming situation. Under varied geo-ecological conditions, the occurrence, distribution and behavioural patterns of malaria vectors and the parasites are known to differ from place to place. The State Orissa is a part of peninsular India with varied geo-ecological conditions which are conducive for transmission of perennial malaria. Complexity and magnitude of malaria in Orissa deserves special attention as the State contributes 15 to 20 % of total malaria in India. About 50 % deaths due to malaria and more than 30 % of falciparum malaria in India are reported from Orissa.

Malaria spreading among tribals has attracted special attention in India. About 22 % of the population of Orissa is tribal. Tribals live in forests, foothills, hilltops and also in plains. There are tribal pockets all over Orissa, mostly in the undivided Districts of Koraput, Kalahandi, Mayurbhanj, Sundergarh, Ganjam and Phulbani, Among all the above Districts, Koraput District has the maximum concentration of tribal population which constitute about 55.22 % of total population of the District. About 75 % of total malaria and 80 % of the falciparum malaria in the State are reported from tribal areas.

This study aims at revealing the relationship between incidence of malaria in tribal and non-tribal PHCs' of Orissa, with special reference to *P. falciparum* malaria. A total of 314 PHCs' (out which 157 Tribal PHCs') of 30 new Districts of Orissa State were taken as the samples of this study. District-wise epidemiological data on malaria prevalence (from the year 1992 to 1996) were collected from Health Departments of the Government. The data analyzed will be presented.

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AN EPIDEMIOLOGICAL STUDY OF MALARIA IN VILLAGE BHANERA, U.P., INDIA

Kumud Sarin
Department of Anthropology, University of Delhi, Delhi 110 007

Malaria is still a serious health problem in India. The epidemiology of malaria is complex, involving factors pertaining to the malaria parasites, the insect vectors, the human hosts and the environment. The environmental and socioeconomic risk factors for malaria were studied in the village, Bhanea, in Ghaziabad District situated near the Hindon river alongwith the objective to acquire epidemiological baseline data. Over a period of one year, all 75 households in the village were visited every alternate day to obtain information on malaria episodes during the cross-sectional survey. Most of the schedules were filled in order to assess socio-demographic variables. Questions were also asked on the presence of clinical signs and symptoms suggestive of malaria during the 3 day prior to interview. Information on risk factors was obtained through questionnaire's and direct observations. The parasite rate was 63.4 % with high degree of parasiteamia. The spleen rate was 27.7 %. So the village was mesoendemic for malaria. Malaria was found in both sexes and in all age groups with marked clustering of cases, but similar infection frequencies by both active and passive detection. The asymptomatic cases exhibited gametocyte stage suggesting that the portion of the village population acts as an reservoir of infection.

Age below 17 years (relative risk (RR) = 1.66, 95 % confidence interval (CI) = 1.118, 2.35), use of bednets (RR = 0.16, 95 % CI 0.05 - 0.45), traditional fumigants (RR = 0.58, 95 % CI 0.37 = 0.93) were independent predictors of malaria. People using anti-mosquito pyrethrum coils had a higher risk for malaria than people living in houses where they were not used (RR = 1.46, CI = 1.03 - 2.07). As regards the treatment modalities, the villagers do not have any faith on the MRC, NMEP and other Govt. officials as they say these people come to the village occasionally and the medicine is not easily available to them. The villages had different perceptions for the causes of disease, some identified as mosquitoes, drinking dirty water, fatigue or hunger. Therefore, the village being near Hindon river and living environmental conditions resulted in the high incidence of malaria.

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EMERGENCE OF MALARIA AS MAJOR PUBLIC HEALTH PROBLEM IN GOA

Ashwani Kumar[1] and V.P Sharma[2]

1. Malaria Research Centre (ICMR), Directorate of Health Services Building, Campal, Panaji 403 001, Goa

2. Malaria Research Centre (ICMR), 20 Madhuban, Vikas Marg, Delhi 110 092

Malaria is transmitted by the well known urban vector *Anopheles stephensi* in Goa. In the recent years, the incidence of malaria has shown upward trend both in the rural and urban areas of the State as the case have increased from 110 in 1985 to over 25000 in 1998. Stratification of Goa based on API and SPR shows existence of two major malaria foci, one in the North Goa District representing Panaji Town, Porvorim (part of Aldona PHC), Candolim PHC and Corlim PHC which account for about 75 % of the total incidence. The second focus exists in the South Goa District representing Margao town, surrounding PHCs and Vasco town which contribute roughly 15 % of the total malaria cases. The remaining urban and rural areas of Goa show variable endemicity of the disease. The proportion of *P.falciparum* malaria has also shown phenomenal increase from just 1 % in 1985 to 33 % in 1998 and the remaining cases belonged to P. vivax. From 1995 to 1998, 86 deaths due to malaria have been reported by the State Health Services Department. Of these, 57 deaths were registered in 1997 alone. This alarming situation warrants urgent drastic antimalarial measures to prevent exacerbation of malaria transmission in the State of Goa where public health is under strain and tourism linked economy could be adversely affected by the disease.

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A COMPARATIVE PROFILE OF ANTIOXIDANT ENZYMES IN SENSITIVE AND RESISTANT FIELD ISOLATES OF *LEISHMANIA DONOVANI*

Anamika Tripathi, Anjna Dixit, Uma Roy and A.K. Rastogi

Division of Biochemistry, Central Drug Research Institute. Lucknow 226 001

Intracellular Leishmanial parasites are constantly exposed to the toxic oxygen metabolites being generated by the invaded immune cells of the host. In order to survive sucessfully in such hostile environment, they possess antioxidant defence mechanism to detoxify the toxic products of oxygen reduction, which include a number of enzymes such as superoxide dismutase (SOD), catalase, peroxidase, glutathione reductase, glutathione oxidase, etc., Emergence of resistance against standard drugs in *Leishmania* strains is a cause of great concern in the recent past, and yet, very little is understood regarding the exact mechanism

underlying the development of resistance. In order to explore the possibilities of any alteration in the levels of the antioxidant enzymes and hence, playing a role in resistance, a comparative study of the these enzymes (SOD, catalase, glutathione reductase, glutathione peroxidase) was done in number of sensitive and resistant field strains of *L.donovani*. WHO reference strain HOM/IN/80/Dd8 which is being regularly maintained in our laboratory was used as a reference strain in the study. The results of the above study will be presented and discussed.

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BIOCHEMICAL DIFFERENTIATION OF SENSITIVE AND RESISTANT STRAINS OF *LEISHMANIA DONOVANI* : A PROTON NMR SPECTROSCOPIC STUDY

U.K. Singha, Anamika Tripathi, Raja Roy and A.K. Rastogi
Division of Biochemistry & Medicinal Chemistry, Central Drug Research Institute, Lucknow 226 001

An estimated 12 million new cases of Leishmanisis occur each year, yet remarkably, very little is know about the metabolism of the causative organism. A comprehensive view of the metabolic capabilities of *Leishmania* spp, in general would be important for three basic reasons : (1) such information may provide insight about various biochemical pathways playing a key role in life cycle, (2) these biochemical pathways, may play some important pathophysilogical role in the host-parasite relationship and could provide targets for innovative therapeutic strategies, and (3) estimation of these metabolities might be useful in diagnosis and identification of its various strains/species. NMR spectroscopy has widely been used as a rapid and convenient *in vitro* method for obtaining comprehensive profile of major intracellular metabolites of parasitic organisms. Hence, in this present study, we have used proton NMR spectroscopic technique and studied comparative and comprehensive profile of intacellular metabilites of sensitive and resistant strains of *L.donovani* promastigotes, suggesting that promastigotes do have a distinct (Finger Print) metabolic profile. The results of the present study will be presented and discussed.

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MONOCYTE FUNCTIONS IN BANCROFTIAN FILARIASIS

M. Aparna, P. Kaliraj, Kuntala Jayaraman and R.B. Narayanan
Centre for Biotechnology, Anna University, Chennai 600 025

Cellular response studies in asymptomatic microfilarics (MF) have shown to be hyporesponsive in terms of Th1 cytokine profile. The level of IL-2, and IFNγ mainly secreted by Th1 are found to be below in MF, whereas in chronic pathology patients Th1

response predominates. Since monocytes form the first line of defense in majority of infections and they release IL-1 which in turn stimulate T-Cells to produce IL-2 for clonal proliferation, it is important to study the role of monocyte in T Cell responses in filarial patients.

Monocytes were purified by two step percoll density gradient centrifugation method and were 95 % positive by esterase and perxidase staining. Normal monocytes were incubated O/N with heat inactivated serum from various clinical categories (MF, CP, EN, NEN) and AB serum. Adherence capacity and phagocytic index of monocytes were assessed. It is observed that the adherence capacity of normal monocytes was significantly reduced when incubated with MF sera as compared to sera from other clinical groups. Phagocytic index however remained similar to that of control. The possible role of certain factors like fibronectin or immune complexes in MF sera is being investigated.

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DIRECT AGGLUTINATION TEST FOR SERODIAGNOSIS OF KALA-AZAR IN INDIA : COMPARISON OF FREEZE DRIED AND AQUEOUS ANTIGENS

Suman Gupta, Suman Tiwari and G.K. Jain*
Division of Parasitology and Pharmaceutics, Central Drug Research Institute, Lucknow 226 001, U.P.*

At present, Direct Agglutination Test (DAT) represents the first line test for diagnosing visceral leishmaniasis in both laboratory and field conditions. Currently, liquid antigen prepared from *Leishmania donovani* promastigotes is used for performing DAT. The aqueous antigen (AQ) has limited shelf-life, requires uninterrupted cold-chain and is heat and shock sensitive. To overcome these limitations, we have developed a new freeze-dried (FD) antigen. It has long shelf-life at ambient temperature, does not require cold chain for storage and transportation and is highly useful for carrying out DAT under field condition. Stability studies have also shown that FD antigen remained fully reactive at 56^0 C for 6 months period covered so far.

FD antigen was evaluated on 282 serum samples from different groups of subjects (Parasitologically proven cases - 50, past treated cases - 20 ; non endemic normals - 75 and patients with a variety of diseases other than kala-azar - 137 (12 with Tuberculosis, 20 with Malaria, 30 with Leprosy, 14 with Amoebiasis, 13 with Hepatitis-B, 42 with Filariasis, 1 with Trypanosomiasis, and 3 with Echinococossis) in comparison to AQ antigen.

With a cut-off value of 1:3200, 47 of the 50 kala-azar proven sera gave positive agglutination with both the antigens, whereas no reactivity was observed with non kala-azar samples. Thus, the sensitivity and specificity of FD antigen was found to be 95 and 100 % respectively - comparing very well with the AQ antigen.

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STUDIES ON ADOPTIVE TRANSFER OF MACROPHAGES IN EXPERIMENTAL MALARIA

C.R. Pillai and C Usha Devi
Malaria Research Centre (ICMR), 22 Sham Nath Marg, Delhi 110 054

Adoptive transfer of purified macrophages harvested from normal, *Plasmodium berghei* infected, latent/cured and also macrophages exposed to parasites *in vitro* were carried out to see the role of macrophages in transfering immunity against *P.berghei* infection.

Macrophages obtained from mice with high parasitaemia at a dose of one million cells/ animal showed significant increase in survival period (SP) and K values, compared to controls. Macrophages exposed to low parasite density conferred significant K values only. There was a decrease in prepatent period (PP) in the animal which received macrophages harvested from animals cured 7-11 months earlier compared to controls.

The adoptive transfer studies with macrophages conditioned *in vitro* to parasite contributed towards increased protection of host against *P.berghei* as expressed by K values only. The adoptive transfer studies showed that the macrophages harvested from infected mice were capable of acting as immunogen against *P.berghei* infection.

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STIMULATION OF NON-SPECIFIC RESISTANCE BY THYMOPENTIN AND ITS ANALOGS AGAINST *LEISHMANIA DONOVANI* INFECTION IN HAMSTERS

Preeti Sharma, Anuradha, A,Rohatgi, W.Haq and J.C Katiyar
Divistons of Parasitology and Biopolymer, Central Drug Research Indtitute, Lucknow 226 001

The visceral form of Leishmaniasis (VL) is a severe and often fatal infection caused by *Leishmania donovani*. The disease is often difficult to treat with the chemotherapeutic armamentarium currently available. In view of the immunosuppression or energy often observed, a reasonable approach to the control of VL would appear to be immunopotention of the infected host. The use of synthetic, chemically defined non - toxic compounds possessing immunomodulatory activity may obviate such problems. Recently, thymopentin (Arg-Lys-Asp-Val-Tyr, TP-5), a pentapetide corresponding to the region 32-36 of the immunostimulating polypeptide thymopoietin has been developed as an effective immunostimulant and found effective in clinical applications in cancer and AIDS patients. The efficacy of TP-5 and its analogs was evaluated at the dose schedule of 3.0 mg/kg and

1.5 mg/kg x 2, i.p. In TP-5 treated group, there was about 48 % inhibition of parasite multiplication at the dose of 1.5 mg/kg. Among the 10 test compounds, Comp. 5(Arg-Lys-Asn-Val-Tyr), 6(Arg-Orn-Glu-Val-Tyr), and 9(DLys-Lys-Hyp-Val-Tyr) exhibited better efficacy (about 52-55 %) than TP-5. These compounds may be further exploited for providing a superior therapeutic regimen in combination therapy along with available antileishmanial drugs.

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IMMUNOPROPHYLACTIC STUDIES AGAINST VISCERAL LEISHMANIASIS WITH ALUM PRECIPITATED KILLED *LEISHMANIA MAJOR* VACCINE + BCG IN INDIAN LANGURS

Alka Misra, Bindu Srivastava, Preeti Sharma, J.K. Sribvastava, Anuradha Dube, J.C. Katiyar and Sita Naik*

*Division of Parasitology, Central Drug Research Institute & *Department of Immunology, SGPGI, Lucknow 226 001*

Autoclaved *Leishmania major* along with BCG, presently undergoing phase II clinical trial by WHO for its vaccine potential against cutaneous leishmaniasis, has been successfully evaluated in single and triple dose schedules against *L. donovani* in Indian langurs (*Presbytis entellus*). Encouraged with these results, another formulation Alum precipitated ALM vaccine (provided by WHO) along with BCG has been evaluated in this system. Eight monkeys were vaccinated with Alum precipitated ALM+BCG (1mg of each vaccinogen per animal) while four were kept as unvaccinated control. All these were challenged with 100 x 10^6 amastigotes i.v. on day 60 post vaccination. Parasitic assessment in splenic tissue was done on day 45, 90 and 180 p.c.. Initially, all vaccinated monkeys developed infection to the tune of 02-14 amastigotes/1000 cell nuclei, which later got resolved by day 180 p.c., when the experiment was terminated. On the other hand, parasite burden in unvaccinated control developed increasingly and three out of four died in between day 110 to day 130 p.c., while one monkey which had low parasite burden survived till the day when experiment was terminated. Prior to challenge, there was an initial rise in antileishmanial antibodies in vaccinated group as compared to unvaccinated control group and later came down to normal level, while it remained higher in unvaccinated control group. An increasing pattern of antigen-specific proliferative responses and interferon-γ (level to the two antigens-ALD and ALM, was observed in vaccinated monkeys throughout the experiment.

The finding suggests Alum precipitated ALM+BCG as a potential vaccinogen against VL and warrants evaluation in human subjects.

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CHARACTERISATION OF *BRUGIA MALAYI* TRANSLATIONALLY CONTROLLED TUMOR PROTEIN (TCTP) CLONED IN *ESCHERICHIA COLI*

M. Gnana Sekar*, K.V.N. Raja Rao*, R.B. Narayanan*, Kunthala Jayaraman*, Alan Scott+ and P. Kaliraj*

** Centre For Biotechnology, Anna University, Chennai 600 025*

+ John Hopkins University, Baltimore, USA

Human lymphatic filariasis is caused by infection with the nematode parasites *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. Most of the immune response studies have been performed using total antigen preparations from *B. malayi* although immune response to a few recombinant antigens have been described. Immune response studies in various clinical groups to individual well characterised molecules would facilitate the understanding of particular antigen(s) involved in immune evasion and the polarisation of host immune responses leading either to protection or pathogenesis. *Brugia malayi* Translationally Controlled Tumor Protein (TCTP) is one of the potential candidate antigen selected in this study. The first described TCTP, p21 from mouse and p23 from human were subcloned and were subsequently shown to cause the release of histamine from basophils in IgE dependent manner. Bm TCTP was obtained from *B. malayi* L4 cDNA library. The clone contains 543 nucleotide open reading frame, encodes 181 amino acid protein. It has 5' conserved Spliced leader, 5' & 3' untranslated region and polyadenylational signal. The coding regions of this gene was cloned into pRSET B vector at *Pam* HI and *Eco* RI sites and expressed as Histidine fusion protein(24.7Kda). The protein was purified by IMAC and the functional studies of BmTCTP are in progress.

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NUCLEAR TECHNIQUES IN PARASITOLOGY

Y.B. Rajashwari

Department of Livestock Production & Management, Veterinary College, Bangalore 560 024

Nuclear energy has been useful in animal parasitology for the production of radiation attenuated vaccines. Study of host parasite interactions and diagnosis of parasitic infections. In pathophysiological studies where the aim to understand how parasites cause disturbances of function in the host with corresponding impairment of production.

Nuclear techniques in conjunction with standard parasitological and immunological methods can play a major role in control of parasites. The development of radio isotopic

methods to elucidate mechanisms by which parasites develop resistance to anti parasitic drugs is also an important aspect to be explored. Radioisotopes have been used to prepare attenuated vaccines. Pathophysiological studies with radioisotopes unravel the mechanisms by which parasites cause disturbances of functions in the host with corresponding impairment or production.

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A CLINICAL TRIAL OF PIPALLI RASAYANA(AN IMMUNOMODULATORY HERBAL DRUG) IN CASES OF GIARDIASIS

Shashi Prabha, S.S. Abbas, A.K. Agarwal* and N. Singh**

** Division of Microbiology, Central Drug Research Institute, Lucknow 226 001*

*** Department of Pediatrics, King George's Medical College, Lucknow*

Pipalli Rasayana(PR) a combined herbal drug prepared from *Piper longum* (Pipalli) and *Butea monosperma* (Palash). Giardiasis is being a very common abdominal problem in Indian population, considered earlier to be of an innocuous nature is now known to cause chronic ill health in many with compromised immune status of its patients, who become prone to catch a variety of other infectious diseases and children suffer from malabsorption syndrome. The modern synthetic drugs like metronidazole are quite effective but do not improve the immunocompromised status of the patient and have many side and toxic effects. The disease being usually water borne, reinfections are common and repeated treatment with synthetic drugs cannot be undertaken without further ill effect of these drugs. Therefore, in the present study PR was selected for trials in 41 cases of stool positive giardiasis. A double blind placebo controlled clinical trial was carried out. In stool positive cases of giardiasis PR was administered in doses of 400mg capsules, one capsule t.d.s for 14 days irrespective of age and weight of the patient. PR significantly reduced the clinical signs and symptoms, stool became free parasites and in haematological profile, Hb gm% increased and eosinophil count decreased significantly. The general health of patients improved and malabsorption syndrome almost disappeared in children. This study shows a clear cut response of PR in the treatment of giardiasis during the 0 to 45 days observations of all parameters studied. No overt side effect of any kind were observed in patients. The dose used was effective in almost all cases. The drug possibly acts through some cidal constituents present in it and also by improving immune status of patients.

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INFLUENCE OF HOST AND PARASITE FACTORS IN EXPERIMENTAL PRODUCTION OF AMOEBIC LIVER ABSCESS

Archana Mishra, Sheela Ghoshal, A.K. Agarwal, H.B Rajani*, P.Y Guru* and B.N. Krishna Prasad

*Department of Microbiology and *Laboratory Animals, Central Drug Research Institute, Lucknow 226 001, U.P.*

One of the three isolates of *E.histolytica* was found to produce amoebic liver abscess in the hamster liver. It failed to produce this condition in other small rodents like rats, mice, mastomys and gerbils. Even among the hamsters, distinct differences were found among the outbred Syrian golden hamster and one of its inbred albinoid variant. The Syrian golden hamsters developed massive amoebic liver abscess with the animals dying out in 3-6 days. Most of the albinoid variant developed restricted abscess and survived the infection for much longer periods.

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ELECTRON MICROSCOPIC STUDIES ON THE ULTRASTRUCTURAL ORGANISATION AT THE ANTERIOR PART OF THE AMASTIGOTES OF *LEISHMANIA DONOVANI*

N.L. Pal, Anuradha, S.C. Maitra and J.C. Katiyar

Parasitology Division and Electron Microscopy Unit, Central Drug Research Institute, Post Box - 173, Lucknow 226 001, U.P.

Electron microscopy in the recent years has equipped us with elaborate knowledge relating to the cytoarchitecture of many intra - and extracellular parasites. *Leishmania donovani,* a causative agent of kala-azar, is one of the important tissue invading haemoflagellates. In man and other vertebrate hosts only non-flagellar stage of the life cycle can develop. Inspite of many cytomorphological studies conducted on the amastigote forms of *L. donovani*, knowledge about its fine structures is still incomplete. Attempts were therefore made to investigate and demonstrate whether the intracellular forms (amastigotes) have any bud of the future flagellum. Studies were also conducted to understand how and when the cell mouth of the amastigotes developed.

Small pieces of spleen from infected hamsters were taken and processed for electron microscopy. Briefly the spleen tissues were placed in small glass vial containing 2% paraformaldehyde, 3% gluteraldehyde in 0.1M cacodylate buffer. When properly fixed, the tissues were dehydrated and embedded in epon araldite mixture. Ultra thin sections of the tissue were cut and examined under transmission electron microscope.

In the spleen sections, hundreds of amastigotes were seen, many of which were lying outside the macrophage. There were intra-and extracellular amastigotes and at different stages of development. In some of these forms a clear cytostome was visible which initially was a cup-shaped depression and later it was transformed into a tabular structure. Within this tube there was a fibrillar complex which appears to be the future flagellum of the next stage of development i.e. promastigote. This paper reports a well defined ultrastructure of the apical organelle which were not described earlier.

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CO-EXISTING FACTORS (SEX, SPECIES, STRAINS AND SEASONS) ON PARASITISM IN LABORATORY ANIMALS

B. Maity and P.Y. Guru

National Laboratory Animal Centre, Central Drug Research Institute, Lucknow 226 001, U.P.

Naturally occured intestinal parasites such as Syphacia, Aspicularis, *Hymenolepis, Giardia, Eimiria, Balantidium, Trichomonads, Chilomastix, Hexamastix spp* were isolated and identified from faecal samples of 8550 adult mice, rats, cotton rats, gerbils, hamsters, mastomys, guinea pigs, and rabbits in our conventional breeding colony over a period of 28 months and established parasitism in relation to sex, species, strains and seasons on above parasites.

It was observed that the males of C_3H/J(*Aspicularis* - 6.54%(M), 2.9%(F); *Trichomonads* - 1.45%(M), 0.36% (F)); SMA (*Hymenolepis* - 10.9% (M), 5.45% (F); *Chilomastix* - 0.72%(M), 0.00% (F)); CF (*Syphacia* - 5.45% (M), 4.49% (F); *Giardia* - 3.27%(M), 0.73% (F) % (F)); DR (*Chilomastix*-0.74%(M), 0.00%(F)); F_{344}(*Giardia*-1.78%(M), 0.72%(F)); Cotton rat (*Giardia* - 1.07% (M), 0.35%(F)); *Hexamastix* - 1.07%(M), 0.35%(F)); Mastomys (*Syphacia*-6.42% (M), 3.15% (F)); Hamster (*Syphacia* - 3.75% (M), 2.85% (F); *Aspicularis* - 3.57% (M), 1.42% (F); *Giardia* - 3.57% (M) 1.42 (F)); Rabbit (BEL) (*Trichomonads* - 1.42% (M), 0.35% (F)); Rabbit (NZW) (*Trichomonads* - 1.09% (M) & 0.36% (F); *Chilometix* - 1.45% (M), 0.72% (F)) were more susceptible to their females on the above respective parasites. Whereas, females of rat CF, DR and Wister were more prone to *Hexamastix, Chilomastix* respectively as compared to their males. It was also observed that the rat DR were more prone to *Hymenolepis spp* as compared to rat F_{344} ($P < 0.05$), Gerbils and almost all strains of mouse respectively ($P < 0.01$). Burden of *Hymenolepis* and *Syphacia* spp were more in mastomys as compared to Gerbils and C_3H/J ($P < 0.01$). *Eimiria spp* infections in rabbit (BEL) were more in comparison to guinea pig and rabbit (NZW), whereas rabbits (NZW) were more susceptible than guinea pigs (F) and rabbit (BEL) (F) to *Giardia* ($P < 0.05$). But the rate of infections of *Balantidium spp* were more in Guinea

pig (both sex) than rabbits (BEL & NZW) ($P < 0.05$). Particular seasonal variation against parasitic infections were not found in this study as the animals were maintained in the strict laboratory conditions through-out the year.

It may be concluded that the host-parasite interactions depend upon the sex, species, strains of the host. Therefore, our statistical significant reports which analysed (test - "Difference between proportions" - Zar - 1974) taking a large sample size in equal environmental laboratory conditions may be considered by the parasitologists to make their best biomedical research protocols.

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STUDIES ON ANTAGONISTIC EFFECTS OF YOGHURT AND ALLIED FERMENTED MILK ON THE ENTEROPATHOGENS

R. Mahalakshmi, G.M. Premalatha and A.M. Natarajan
Department of Microbiology, Maharani's Science College for Women, Bangalore 560 001 And R & D Laboratory of K.C. Das Pvt. Ltd, Bangalore

Importance of dietary changes to optimize health is gaining recognition and importance. In this connection, milk and milk products occupy a significant place in our day to day food, specially fermented products like curds, yoghurt, cheese etc. Milk offers a condusive medium for microbial growth. Synergy of nutritional and health benefits obtained from varied microbial strain would appear to open a large scope for food biotechnologists in the immediate future. Thus milk is an excellent medium to generate an array of dairy products that fit into current consumer demand for health driven food. Yoghurt is a fermented milk product especially yoghurt obtained through controlled lactic acid fermentation of milk using symbiotic blend of starter cultures comprising *L. bulgaricus* and *S. thermophilus* in the ratio of 1:1. This product is very common around the world, claimed to be possessing both therapeutic and nutritive value. Yoghurt is regarded in the most popular literature as a source of Lactobacilli that can transmit beneficial effects to the intestinal tract. It is claimed that large quantities of cultured yoghurt lowered serum cholesterol, inhibitory action on specific tumour cells and limit the course of diarrhoel disease. Yoghurt is also marketed in the form of Misti dahi which involves use of yoghurt cultures, grown in concentrated milk with 20% sucrose. In order to assess the antagonistic effect, apart from possessing the above attributes, an antibiogram assay was carried using pathogenic strains viz. *S. aureus, E. coli* and enterotoxigenic strain of *B. cereus* as seed culture against the Plain and Misti dahi filtrate solution. Plain yoghurt and Misti dahi yoghurt exhibited zone of inhibition on strains of *E. coli, S aureus* and enterotoxigenic strains of *B. cereus*. The plain yoghurt filtrate exhibited clear zone width of 45 and 48 mm for *E. coli* and *B. cereus* while concentrated filtrate gave wide zone of 50 and 53 mm respectively. The zone width observed was more in case of Misti

dahi compared to plain yoghurt against both the enteropathogens. To obtain a rich harvest of yoghurt culture as well as their appropriate ratio of *B. thermophilus* and *L. bulgaricus* needed for production of maximum antagonistic effect, a method of its manufacture has been standardised.

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TWO NEW SPECIES OF *HEMICRICONEMOIDES* (NEMATODA : TYLENCHIDA) ASSOCIATE WITH LITCHI FRUIT TREES FROM DOON VALLEY, (U.P) INDIA

Nisha Srivastava, Vikram S. Rawat and Maqsood Ahmad
Nematology Laboratory, Zoology Department, Government. P.G.College, Rishikesh, Dist. Dehradun, U.P.

Two new species *Hemicriconemoides neoabberans* n.sp. and *H.doonensis* nematodes belonging to class Tylenchida are first time recorded around the root zone of Litchi fruit trees from Doon Valley. *H. neoabbearns* n. sp resembles with *H. abberans* (Phukan & Sanwal, 1982) and *H.mangiferae* (Siddiqi, 1961) but differs in having expanding cuticular sheath with three annules, vulval flaps with three annules, long stylet, excretory pore just behind oesophagus (cuticular sheath with 2 annules and vulval flaps absent, stylet = 66-78 µm; R = 122-144; Rv = 13-14; Ran = 7-10; Rex = 29 in *H.abberans*) and short body length, longer tail, more annules in *H.mangiferae, H.doonensis* n. sp resembles with *H. virablis* (Rahman & Ahmad, 1995) and *H.variodus* (Choi et Grootaert, 1972) but differs in having two lip annules, labial plates modifie to semicircular sheath like projection, stylet long and tail conical (stylet = 85-95µm; tail = 26-32µm in *H.virablis*) and spear knob with sloping anterior surface, short oesophagus, long pointed tail and posterior vulval annules more (Oesophagus = 185µm; tail = 51µm; v = 91-93; Rv = 11-15 in *H.vironoudus*).

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ISOENZYME TYPING OF *LEISHMANIA* PARASITE OF DIFFERENT GEOGRAPHICAL DISTRIBUTION IN BIHAR, INDIA

C S.Lal, A. Palit, A.K.Gupta, N.K.Sinha, R.Singh, N.Verma, V.N.Ravidas and S.K.Kar
Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna 800 007 Bihar

The recent resurgence of visceral leishmaniasis in Bihar and further, the scenario is complicated by the unresponsiveness to drugs, this trend suggest the need to understand various epidemiological factor including strain differences in *Leishamania* parasite. Keeping in view the above facts, this project has been undertaken.

Twelve leishmaniasis isolates (parasitologically confirmed as VL) has formed the study group while WHO reference strain of *L. donovani* (MHOM/IN/80/DD8) has been taken as control. All these strains were grown in a modified Tobie's medium and pure clonal culture were prepared by serial dilution and passaging. Further, the extracts were prepared according to Kruetzer & Christensen (1980).7.5% polyacrylamide was used in PAGE and method essentially described by Davis (1964) was followed. The enzyme studied were GPI,LDH,MDH,ME,SOD,ALDH,ADH and PGM. The gels were visualized for enzymes and their electrophoretic mobilities were calculated as described by Katoch *et al* (1986). The zymograms based on these F values of the bands of individual strain were constructed and analysed for species and strain differentiation. All isolates so far obtained have similar isoenzymes profile as DD8 (*L.donovani*). It may be noted that the PKDL strains and the unresponsive strain isolated from a patient unresponsive to SAG failed to show any intraspecies difference from control DD8. The study at this preliminary stage tentatively concludes that no major strain variation has taken place in this geographical region. However, it is postulated that more number of isoenzymes will pave way for more species specific enzymes in larger number of *Leishmania* isolates. The details will be discussed.

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CESTOCIDAL ACTIVITY OF PECTIN ISOLATED FROM *CARICA PAPAYA*

R.Dutta (Nag), S.P.Sinha Babu and N.C.Sukul
Department of Zoology, Visva-Bharati University, Santiniketan 731 235, W.B.

Pectin, a known polysaccharide obtained from *Carica papaya* L. (Caricaceae) was administered orally to a batch of 10 albino rats infected with *Hymenolepis diminuta* at 500mg/kg body weight for 10 days. The rats were starved for 24h before treatment. The average egg count decreased from 59,941 before treatment to 14,612 per animal on day 13 following the onset of treatment. There was no appreciable change in the mean egg counts in the control rats. Treatment of another batch of infected rats on days 4 and 6 post inoculation (PI) with the same drug at 500mg/kg body weight followed by necropsy on day 15 PI resulted 54% clearance of the parasite burden from the host. The present results reveal that pectin possesses strong cestocidal activity against *H. diminuta* in rats.

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CESTOCIDAL ACTIVITY OF *SAPINDUS DETERGENS*

N.K. Ghosh, S. P. Sinha Babu and N. C. Sukul
Department of Zoology, Visva-Bharati University, Santiniketan 731 235, W.B.

Ethanol extract obtained from the fruits of *Sapindus detergens* (Sapindaecae) was administered orally to a batch of ten albino rats infected with *Hymenolepis diminuta* at 300mg/kg body weight for 3 days. The rats were starved for 24 h before treatment. The average number of discharging eggs decreased significantly after treatment. There was no appreciable change in the mean egg counts in control rats. Treatment of another batch of infected rats on day 4 PI with the same drug at 400mg/kg body weight followed by necropsy on day 15 PI resulted 75% clearance of the parasite burden from the host. The present results clearly indicate that the ethanol extract of *Sapindus detergens* possesses strong cestocidal activity against *H. diminuta* in rats.

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SOME STUDIES ON FAMILY LONGIDORIDAE THORNE, 1935 (NEMATODA:DORYLAIMIDA) FROM GARHWAL HIMALAYAS (U.P.), INDIA

Maqsood Ahmad, Vikram Singh Rawat and N.Srivastava
Nematology Research Laboratory, Zoology Department, Government P.G.College, Rishikesh 249 201, U.P.

A systematic survey of plant-parasitic nematodes during 1995-98 from five hill districts of Garhwal Himalayas (U.P.) India at different altitudes yielded about 80 populations of the genera *Xiphinema, Longidorus* and *Paralongidorus* belonging to the family Longidoridae (Thorne, 1935). Many species like *Xiphinema americanum, X.inaequale, X.insigne, X.elongatum, Longidorus altenuatus, L. africanus, L. reneyii* and *Paralongidorus sali* were identified. Two new species, one each in the genera *Xiphinema* and *Longidorus* have been proposed. These are illustrated and described in detail. Most of the populations in the genus *Xiphinema* belonging to *X. americanum* group, a most complex and diversified group. *X. inaequale* was most frequent at all altitudes. The morphometric study of about 20 populations of *X. inaequale* at different altitudes/climate was conducted and it was concluded that the body length, odontostyle, odontophore, guiding ring, tail length and shape were most variable characters. These were co-related with different altitudes/climate regimes.

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TWO KNOWN AND ONE NEW SPECIES OF THE GENUS *MONONCHUS* (NEMATODA:MONONCHIDA) IN GARHWAL HIMALAYAS (U.P.), INDIA

Vikram Singh Rawat*, N. Srivastava and Maqsood Ahmad
Nematology Research Laboratory, Zoology Department, Government P.G.College, Rishikesh 249 201, U.P.

** c/o Shri Roshan lal Sharma, Baba Kali Kamli, Rishikesh (Dehradun)*

Two known and one new species of the genus *Mononchus* have been described. *M. aquaticus*, *M. truncatus* and *M. himalayensis* n.sp. were collected from Garhwal Himalayas. *M. truncatus* is reported for the first time in India from Garhwal Himalayas.

M. himalayensis n.sp. comes close to *M. truncatus* (Bastian, 1865) and *M. pulcher* (Andrassy, 1993). It differ from the former in the value of "c", apex of dorsal tooth from the anterior side of stoma and tail length. From *M.pulcher* it differ in having longer oesophagus, longer buccal cavity, tail length and in the value of "c" and "c'".

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STUDIES ON THE CELLULAR DEFENSE MECHANISM IN SILKWORM, *BOMBYX MORI* L.

B.R.Jamuna and C.S. Patil
Silkworm Pathology laboratory, Karnataka State Sericulture Research and Development Institute, Thalaghattapura, Bangalore 560 062

The studies were conducted on changes in haemocytes and their proportion percentages in silkworm, NB_4D_2 race from III instar to the end of larval period under the non-infective conditions. It was revealed that granulocytes (67.7%), spherulocytes (12.8%) and plasmotocytes (10.90%) were high in number while oenocytes (2.3%) and prohaemocytes (0.3%) were in low proportion to their total haemocyte numbers. Variations in the total haemocyte counts (THC) during developmental stages of silkworm were recorded.

The sequential host parasite interaction were studied by injecting the live *Echerichia coli* cells (10^4 cells/larva) into the haemocoel of silkworm. As a defensive reaction, there was an immediate changes in THC in the host. Bacterial cells were found adhered to granulocytes within minutes of injection and were phagocytosed and decrease in the free circulating bacteria was observed. The process of formulation of nodule by haemocytes as defensive mechanism against 10 fold increase in bacterial challenge number are discussed.

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EFFECT OF LEAF EXTRACT OF *AZADIRACHTA INDICA* ON ENZYMES OF CARBOHYDRATE METABOLISM OF *TRICHURIS GLOBULOSA* (NEMATODA)

Santosh Gandhi*, Rashmi Sarwal and Sarika Kanwar
** Department of Zoology, Punjab University, Chandigarh 160 014*

Effect of ethanolic neem leaf extract (prepared in soxhelet apparatus) was studied on some enzymes of carbohydrate metabolism in *Trichuris globulosa* (v.Linstow). The enzymes, namely, Glucose-6-Phosphatase (G-6-Pase), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) were assayed in their respective buffers and they showed a typical Michaelis-Menten substrate saturation kinetics. V_{max} (maximum of the apparent enzyme velocity) and K_m (substrate affinity constant) were calculated directly from the plots.

The activity of the enzymes was shown to be effectively inhibited by the *in vitro* addition of the neem extract to the enzyme assay system at a concentration of 0.5% (LD_{50} was calculated of using different concentrations of the neem extract).

ACP activity was inhibited in an un-competitive nature as both the V_{max} and the K_m were lowered i.e. the drug affects the substrate utilisation by the enzyme.

ALP activity was inhibited as that of ACP i.e. un-competitive nature where both the V_{max} and the K_m values were lowered.

G-6-Pase activity was inhibited in a non-competitive manner in which both the substrate utilisation and the enzymes molecules were affected i.e. V_{max} was lowered and K_m remained unchanged.

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OCCURRENCE,MORPHOLOGY AND ABUNDANCE OF METACERCARIAL CYSTS OF *MICROPHALLUS* SP. INFECTING PENAEUS *INDICUS* H. MILNE EDWARDS

R. Jayakumar and P. Ramasamy
Department of Zoology, Life Sciences Building, University of Madras, Guindy Campus, Chennai 600 025

A survey was carried out on the occurrence and abundance of encysted metacercariae of *Microphallus* sp. infecting *Penaeus indicus* obtained from Ennore estuary, Chennai, India. The study revealed the occurrence of metacercarial cysts of *Microphallus* sp. in pleopods, uropods, gills, exoskeletal structures and hepatopancreas of *P. indicus*. The cyst of

Microphallus sp. was spherical in shape and consists of two layers of cyst wall with an outer layer and a thin transparent inner layer. Brown to black colour formation, accumulation of haemocytes and chromatophores were noticed in the vicinity of *Microphallus* sp. infected tissues. Prevalance of cysts of *Microphallus* sp. ranged from 3.8% to 94.4% and the abundance was 12 cysts/host of *P. indicus*. The cysts were overdispersed in the population of *P. indicus*. The observed frequency distribution of the cysts fitted well with negative binomial model than with poisson.

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CHARACTERIZATION OF HETEROCHROMATIN IN THE CHROMOSOMES OF SOME MOSQUITOES

G. Venkatachalaiah and R. Prasanna Kumar
Centre for Applied Genetics, Department of Zoology, Bangalore University, Bangalore 560 056

The present study intends to provide cytological description of mitotic, meiotic and polytene chromosomes in response to various chromosome differential staining techniques applied on some representative mosquito genomes.

Major emphasis is placed in the genomes of *Anopheles stephensi, An. nigerrimus, An. fluviatilis* (Anophelines), *Aedes aegypti, Ae. albopictus* (Aedines), and *Culex quinquefasciatus* and *C. tritaeniorhynchus* (Culicines), all belonging to the family Culicidae. In order, to delineate longitudinal differentiation of metaphase and meiotic chromosomes, classical G-, Q- and R-banding procedures and for scrutinizing compositional heterogeneity, C-, N- and $AgNO_3$ staining were used for detailed analysis.

Our attempts to highlight the description of cytological entities in a chronological order during various developmental stages to distinguish between male and females, have yielded distinctive results for both anopheline and cuclicine mosquitoes, more so from meiosis. Attempts were also made further to characterize composition of heterochromatin in the genomes and thus chromosome morphology in response to *in vivo* effect of some DNA ligands (such as BrdU, Ethidium Bromide, Acridine Orange and Hoechst 33258) on the embryonic and adult tissues and subsequent staining by appropriate dyes have facilitated in eliciting differential structural organization and their subsequent behavioural manifestation in each species was recorded and was used for generalization.

Fixed metaphase chromosomes were when treated with Restriction Endonucleases (RE), followed by Giemsa staining, revealed several substructural organization, especially among culicine genomes. Alu I, Hinf or Mbo I treatment produced a C-band pattern and Eco II or Hae III produced G-band plus C-band pattern. Ava II and BSt NI each produced

a G-band pattern but most only a small segment of each C-band adjacent to centromere was stained. These tiny residual C-bands may contain a minor satellite located adjacent to the major satellite clusters are evident with Anophelines but not in Culicines.

The results found with individual chromosomes in the different species appear relevant in the light of the evolutionary relationship between these two broad chromosomal groups within mosquitoes. Lastly, the results suggest that the presence of some highly repetitive DNA sequences in anophelines fundamentally different in their genomic compaction from the other culicine mosquito genomes, including polytene organization.

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ENDOXAN INDUCED CHROMOSOMAL ABERRATION IN SOME MOSQUITO GENOMES

J.C. Badarinath, Jayaprakash and G. Venkatachalaiah
Centre for Applied Genetics, Department of Zoology, Bangalore University, Bangalore 560 056

Genotoxic potential of the antitumour therapeutic agent and a mutagen, Cyclophosphamide (endoxan) was tested on the mitotic, meiotic and polytene chromosomes of *Anopheles stephensi, Culex, quniquefasciatus* and *Aedes albopictus* against suitable controls by chromosome aberration test. Application of predetermined sublethal concentration in three different exposures showed that endoxan could induce chromosome and chromatid fragmentation, gaps and yielded a few plates with aneuploid constitution. The effect appeared more severe on the paracentromeric and heterochromatic regions in the complement, especially so in anopheline species.

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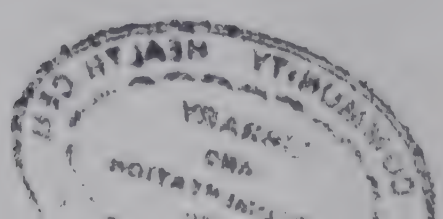
LARVAL TREMATODES INFECTING SNAILS IN FRESHWATER TANKS OF WEST GODAVARI DISTRICT, ANDHRA PRADESH

P. Krishna Mohan[1] and G. Gnana Mani[2]
1 Dr. K.S. Raju Degree College, Penugonda, West Godavari Dist., A.P.
2 Department of Zoology, Andhra University, Visakhapatnam 530 003

A survey of larval trematodes in freshwater gastropods of West Godavari District in Andhra Pradesh was undertaken during October 1987 to September 1988. Four species of snails viz, *Lymnaea luteola, Indoplanorbis exustus, Bellamya bengalensis* and *Digoniostoma cerameopoma* were collected and examined. Of the 11 species of cercariae encountered, three species were of zoonotic importance. These are cercariae of *Echinostoma lindoensis*, *Echinostoma malayanum* and *Schistosoma spindlae*. The morphology and ecology of the



larval forms is discussed in the light of their zoonotic potential. The need for control of snail vectors in the freshwater bodies is highlighted.

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ROLE OF LABORATORY ANIMALS IN PARASITIC DISEASES

Y.B. Rajashwari and K. Sathyanarayan*
Department of Livestock Production and Management, Veterinary College, Bangalore 560 024

The use of laboratory animals in the field of scientific investigations is of respectable antiquity dates back to some centuries. However, it was sporadic limited and animals used were only domesticated animals. Animal experimentation become particularly useful and rewarding when diseases due to parasites and microbes could be established and reproduced in small animal species. The parasitic infection affecting man and livestock are Amoebiasis, Leishmaniasis, Trypanosomiasis, Malaria, Helminthiasis and Ectoparasitic infections are of paramount importance since these diseases cause the major impediments to the socio-economic progress.

Disease	Animal model	Purpose
Protozoan infections		
Amoebiasis	Rats, Syrian Hamsters	Demonstration of the organisms
Trypanosomiasis	Rabbits, guinae pigs, Rats and Mice	To induce infection
Leishmaniasis	Dogs, Chinese Hamsters European Hamsters Cotton Rats	Act as laboratory hosts
Trichomonad infection	Rhesus Monkey	To establish and to induce infection
Malaria	Rabbits, Hamsters and Mice Chimpanzy, Monkey	Experimental infection
Trematode infection		
Schistosomiasis	Rabbits, Guinea pigs,	To induce infection
Nematode infection		
Ascariasis	Pigs	For the development of newer anthelminthics
Hookworm	Hamsters	For the development of newer anthelminthics
Filariasis	Cats	Experimental establishment of the organism

Ectoparasites Acarine Ticks	Rabbit	To obtain different developmental stages in the life cycle.

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ON THE SYNONYMY OF *GANEO ATTENUATUM* SRIVASTAVA, 1933 WITH *GANEO TIGRINUM* MEHRA ET NEGI 1928

H.C.P. Sah and U. Sahay

Postgraduate Department of Zoology, Ranchi University, Ranchi 834 009

G. tigrinum was described by Mehra et Negi, 1928 which they recovered from *Rana tigrina* at Allahabad but another trematode *Ganeo attenuatum* was described by Srivastava, 1933 from *Rana cyanophlyctis* at Sitapur (U.P). Simha, (1958) reported the ocurrence of *G. tigrinum* in *Chamaeleon zeylanicus*.

The authors have compared and discussed the two species in detail and have reasons to consider *G. attenuatum* to be a synonym of *G. tigrinum*. The parameters considered for this synonymy are length of the worms, oral sucker, ventral sucker, pharynx, oesophagus, testes, ovary, extension of vitellaria, excretory vesicle, receptaculum, seminis, vesicula seminalis and metraterm.

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PHILOMETRA INDICA N.SP. FROM THE BODY CAVITY AND GONADS OF FRESH WATER FISH *CHANNA PUNCTATUS* (BLOCH) OF VISAKHAPATNAM

M. Sudha and B. Bharatha Lakshmi

Department of Zoology, Andhra University, Waltair

Philometra indica, a new species from the body cavity and gonads of *Channa punctatus* (Bloch) of Visakhapatnam is described. The worms are elongated, filiform and red in colour. Anterior and posterior extremities rounded. Mouth, simple without lips or papillae. The present species resembles *P. channai* in the absence of lips or papillae, but differs in having ventriculus at posterior end of oesophagus, position of ovaries, structure of female tail. The present species differs from *P. pellucida* in the absence of cephalic papillae or lips. In the light of these differences, the present species in considered as new species and named *philometra indica*. Visakhapatnam is a new locality record for the genus *Philometra*.

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A NEW SPECIES OF *RHABDOCHONA* (NEMATODA) FROM A MARINE FISH *LINOPHORA VAGABUNDA* OF POODIMADAKA

M. Sudha and B. Bharathalakshmi

Department of Zoology, Andhra University, Waltair

Rhabdochona vagabundai, a new species of the genus *Rhabdochona* from the intestine of marine fish *Linophora vagabunda* is described. The present species closely resembles and agrees with some characters of *R. barbi* in having cervical papillae, non-alate spicules, boat shaped right spicule and expanded and truncated left spicule. But differs in body size, number and arrangement of caudal papillae, length of spicules, presence of narrow caudal alae and having left spicule with a spine. The present species resembles *R. paxmani* in having left spicule with spine but differs in the number of longitudinal ridges supporting buccal capsule, right spicule devoid of barb and spicular ratio 1:2 versus 1:4.3 in *R. paxmani*. In view of these differences, the present species is considered as new and named as *R. vagabundai*. Poodimadaka is new locality record for the genus *Rhabdochona*.

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REGULATORY MECHANISM OF THE MALE ACCESSORY REPRODUCTIVE GLAND (ARG) OF *SERINETHA AUGUR* (FABRICIOUS) (HETEROPTERA : COREIDAE) - A COTTON PEST

P. Basker [1] and L.S. Ranganathan [2]

1 Institute of Vector Control & Zoonoses, 150, Sipcot Complex, Hosur 635 126

2 Department of Zoology, Annamalai University, Annamalai Nagar 608 002

The present study deals with the inter-relationship of the neuroendocrine complex (NEC) and male accessory reproductive gland (ARG) of a cotton bug, *Serinetha angur*. The NEC consists of brain (cerebral ganglia), corpus cardiacum (CC) and corpus allatum (CA). Based on the staining reaction of aldehyde fuchsin (AF) and chrone alum haematoxylin - phloxin (CAHP), four types of neurosecretory cells have been identified in the brain. The neuroendocrine control over the ARG in *S. augur* was investigated through ARG extirpation (and gonadectomy) induced hypertrophy of CA. The changing pattern of proteins in the brain during the pre and post mating period (as judged by electrophoretic investigations) further supports the interrelationship of the NEC and ARG.

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EGG-FLOAT RIDGE NUMBER IN *ANOPHELES STEPHENSI*: ECOLOGICAL VARIATION

B.H. Rajasree and N.J. Shetty
Centre for Applied Genetics, Bangalore University, Jnanabharathi Campus, Bangalore 560 056

Anopheles stephensi is one of the important malaria vectors in the Indian subcontinent. Taxonomically, it is a member of sub-genus *Cellia* and series Neocellia

The strategy for vector control vary according to the vector species and depends upon the fundamental knowledge of their biology, ecology and ethology. Specific ridge number on the egg float of *An. stephensi* has made it possible to classify the species into three forms viz., *type* (14-22 ridges), *Mysorensis* (9-15 ridges) and *intermediate* (12-17 ridges).

In the present investigation, twelve strains of *An. stephensi* were tested for their egg-float ridge number. Most of the regions that were surveyed consisted of urban and semi-urban localities. The strains of Geruguntepalya and Kengeri were grouped under type form with 15-19 and 16-22 ridges respectively. The strains from Rajajinagar, Thalaghattapura, Yelahanka, T.Nagar and Chennai and Pune were grouped under intermediate with 13-18, 11-18, 12-18, 13-18 and 12-14 ridges respectively, Chetpet of Chennai and Mangalore strains were grouped under variety *Mysorensis* with 11-14 and 10-13 ridges on the egg-float respectively. The rest of the strains i.e Cubbonpet, Delhi and Pondicherry had both *intermediate* and *Mysorensis* forms with 10-16, 10-18 and 10-16 ridges respectively. From the data, it is clear that all the three forms of *An. stephensi* have been identified in the strains included in the present investigation. It is interesting to note that in the present study, the *type* form has been traced from the semi-urban areas occupied by the population of high income group. Similarly, *Mysorensis* variety was traced from the urban localities surrounded by slum dwellings. This is in contrast to the earlier observations where, the type form, an efficient vector of malaria was reported to be prevalent in the urban areas, and the *Mysorensis* variety, a poor vector was present in rural areas. However, it is observed that the high ridge number *type* form was not traced in the rural localities which is in agreement with earlier reports. It was observed that the strains of Cubbonpet (Bangalore), Delhi and Pondicherry consisted of both *intermediate* and *Mysorensis* forms. The ridge number in Delhi strains was in agreement with earlier reports.

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VECTOR COMPETENCE OF NONBITING FLIES IN A RURAL AREA NEAR MADURAI

S. Jonathan Nimal and R. Selvaraj Pandian
Postgraduate & Research Department of Zoology, The American College, Madurai 625 002

Vector and nonvector nonbiting flies occur more predominantly in the rural areas and are responsible for many enteric and other diseases. Among these flies, house fly is more prevalent and acts as mechanical vector for several enteric diseases. Four main sites were selected based on the aggregation of human for foraging activity. The flies were collected by using sterile containers and then transferred to sterile saline solution immediately. Fly dipped solution was serially diluted and the microbes were cultured in differential and specific media. In addition, biochemical tests were also carried out to confirm the microbes at generic and species level or both. Isolated microbes were kept in sterile slants and preserved. *Staphylococcus* spp, *Streptococcus* spp, *Salmonella* spp, *E coli* and other microbes were identified. Presence of pathogenic and non pathogenic microbes in the present study from the body wash of housefly indicates its vector competence and therefore it needs proper management of flies in order to contain this vector and subsequently the disease transmitted by them.

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ON A NEW SPECIES OF GENUS *CIRCUMONCOBOTHRIUM*, SHINDE, 1968 FROM *MASTACEMBELLUS ARMATUS*

S.V. Kharade and G.B. Shinde*
Department of Zoology, Krishna Mahavidyalaya, Shivanagar, Tal. Karad, Dist Satara 415 108

** Department of Zoology, Dr. B.A. Marathwada University, Aurangabad 431 004, M.S.*

The genus *Circumoncobothrium* was erected by Shinde, 1968, with its type species *C. ophiocephali*, collected from *Ophiocephalus leucopunctatus*. The present communication deals with the morphology of a new species *C. mastacembellusi* collected from, freshwater fish, *Mastacembellus armatus*. *C. mastacembellusi* n.sp. is distinct from other species of the genus *Circumoncobothrium*. The species is characterised by scolex, large, tapering at both ends, broad in middle; restellum oval, armed, rostellar hooks 38 in number, arranged in four quadrants, rod shaped, two types, longer in centre, shorter on both sides; two bothria, large, almost triangular, narrow anteriorly, broad posteriorly, occupying almost whole region of scolex. Neck is absent.

Mature segments, thin, broader than long, almost 17 times broader than long, irregular,

Life fertility table was prepared according to Birch (1948) and life table statistics were calculated according to Laughlin (1965). Age specific survival curves were drawn as suggested by Slobodkin (1962).

N. thymus exhibits sexual dimorphism and is parthenogenetic producing males parthenogenetically. Average realised fecundity was 309.93±30.97 and longevity was 17.90±0.87 days and 19.50±1.35 days for males and females respectively. However, longevity decreased in the absence of host. Period of intensive egg laying was not obvious throughout its life span. Net reproduction rate was 235.148, intrinsic rate of natural increase was 0.2381, and finite rate of increase was 1.2688. Generation time for *N. thymus* was 22.84 days and it took 2.89 days to double itself.

Survivorship curve indicated that it followed a type III curve of Slobodkin, indicating that the rate of mortality was constant throughout its lifespan.

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HOST SPECIFICITY OF THE PARASITOIDS OF UZI FLY *EXORISTA BOMBYCIS* (LOUIS), A SERIOUS PEST OF SILKWORM *BOMBYX MORI* L.

H.K. Jyothi, Geetha Bali and G.veeranna

Host specificity studies were conducted on six hymenopteran parasitoids of uzi fly *Exorista bombycis* (Louis) (Diptera : Tachinidae), a serious pest of mulberry silkworm *Bombyx mori* L. (Lepidoptera : Bombycidae), in an attempt to short list candidates for field release and also to test safety to benefical dipterans when releases were made.

Three hymenopteran ectopupal parasitoids namely, *Nesolynx thymus* (Girault) (Eulophidae), *Pachycrepoiedeus veerannai* (Narendran and Anil (Pteromlidae) and *Dirhinus anthracia* Walker (Chalcididae) and three endopupal parasitoids namely *Trichopria* sp (Diapriidae), *Exoristobia philippinensis* Ashmead (Encyrtidae) and *Brachymeria lugubris* (Walker) (Chalcididae) were tested for host preference on 6 economically important dipteran pupae. Preference was measured using methods suggested by Wilson (Picket *et.al.*, 1989) and Chesson (1978).

Results indicated that *N. thymus*, was a generalist followed by *E. philippinensis* and *Trichopria* sp. *Dirhinus anthracia* exhibited highest preference for *E. bombycis* pupae.

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ANTHELMINTIC ACTIVITY OF LAWSONE ON *FASCIOLA HEPATICA*

Laxmikant Dama and B.V. Jadhav
Department of Zoology, Dr. B.A. Marathwada University, Aurangabad 431 004, (M.S)

Lawsone (2-Hydroxy-1, 4-Napthoquinone) was tested *in vitro* against liverflukes (*F. hepatica*). Although *in vitro* activity was found on the motility of *F. hepatica* at concentrations 100, 200 and 300 µl / 200 ml Rohrbacher's enriched medium, was 33.3, 60.0 and 93.3%. The lawsone inhibit the motility of *F. hepatica*, this spectrum of activity suggests that the lawsone shows anthlmintic activity.

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PREVALENCE OF LIVER FLUKE CERCARIAE IN THE IMMATURE STAGES OF *ANOPHELES FLUVIATILIS* AT DIFFERENT ECOLOGICAL CONDITIONS

B.P. Pokhriyal and R.K. Jauhari
Parasitology Laboratory, Department of Zoology, D.A.V. (P.G) College, Dehra Dun 248 001

The results of the present investigations are based on the adaptation of liverfluke cercariae in different larval stages of *Anopheles fluviatilis* at different water temperatures, light conditions and pH of the water. The prevalence of cercariae was maximum in 4th instar stages at 24 and 40^0 C, whereas in 3rd instar it was at 32^0 C. The pupae also exhibited antagonsim efficiency at all the three levels of tempratures but to a less extent in comparison to 2nd, 3rd and 4th instar larvae, There was no response of 1st instar larvae towards the cercariae at all the intensity level of light conditions. The maximum prevalence was in 3rd instar larvae and that too in sharp light conditions. Whereas, in dim light and darkness the maximum prevalence was observed in 4th instar larvae. At both the levels of pH i.e, 6.5 and 7.5 the maximum prevalence was seen in 4th instar larvae followed by 3rd instar.

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PROTEIN PROFILES OF EGG-SHELL OF *FASCIOLA GIGANTICA*

P. Balasubramanian and P. Ramasamy
Department of Zoology, Life Sciences Building, University of Madras, Guindy Campus, Chennai 600 025

The uterine tubules of the *Fasciola gigantica* were dissected out from freshly collected specimens. Eggs were isolated from the uterine tubules. They were homogenized and the h[illegible]genate was centrifuged at 5000 rpm for 15 minutes at 4^0 C. The pellet containing

broken pieces of egg-shell was washed three times in saline medium. Further, the broken pieces of the egg-shell were sedimented and purified by sucrose density gradient centrifugation (40 to 80%) at 3000 rpm for 30 min. The purified pieces of egg-shell were subjected to sodium nitroprusside, 10% lead acetate and β-mercaptoethanol (to break the S-S and S-H linkages), sodium thiosulphate, sodium dodecyl sulphate, tris-glycine buffer and triton X-100 and then the protein profiles were determined. The tris-glycine extract of the egg-shell revealed the occurrence of 18 subunits of proteins. Three of them were low molecular weight proteins (26, 25 and 23 kDa) and formed the major components of the egg-shell. Alkali treatment of the broken purified pieces of egg-shell with nitroprusside and ammonium hydroxide resulted in the generation of only a low molecular weight subunit approximately < 1400 DkDa. All the protein fractions obtained from the egg-shell of *F.gigantica* are negative to PAS reaction and toluidine blue and these may indicate that the egg-shell proteins are free from muco-and glyco moieties.

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PERMEABILITY PROPERTIES OF EGG-SHELL OF *FASCIOLA GIGANTICA*

P. Balasubramanian and P. Ramasamy
Department of Zoology, Life Sciences Building, University of Madras, Guindy Campus, Chennai 600 025

The egg-shells of parasitic helminths of trematode protect the life of the developing embryo. The egg-shell precursor proteins are synthesized and stockpiled in the vitelline cells, stabilized by S-S and S-H linkages/quinone tanned in the ootype of mature *Fasciola gigantica*. Properties of egg-shell as well as the chemical make up including linkages of the reactive groups which may have a bearing with stability as well as the preponderance of the type of linkages. Solubility studies may give clues in the understanding of the nature of the cross-links that determine the three dimensional architecture of structural proteins. For example, alkali hydrolysis of keratin break of disulphide cross-linkages. Collagen dissolves in 2% acetic acid. In view of these, the egg-shells of *F. gigantica* were subjected to various acids, alkalis and chemicals. The cross-linkages of a seal of a proteinaceous cement material hold the operculum in place and this opercular cement was dissolved when the eggs were exposed to 0.1N and saturated NaOH, concentrated HCl, H_2SO_4 and H_3PO_4 , whereas the rest of the egg-shell remained unaffected. TCA, H_3BO_3, CH_3COOH did not affect the quinone tanned egg-shell as well as the linkages of the opercular cement. Concentrated HNO_3 and NaOCI solubilised the egg-shell by cleaving quinone and S-S linkages. This unhardening process exposed the reactive groups of the proteins of the stabilised egg-shell. This study has shown that the operculum cement, the operculum and the remaining part of the egg differ in their permeability properties. The application of the technique of dissolution of the opercular ce-

ment and egg shell in interfering with the life cycle of *F. gigantica* remains to be exploited.

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ANTIFILARIAL EFFECT OF A COMBINATION OF BOTANICALS FROM *CENTELLA ASIATICA* AND *SANSEVIERIA TRIFASCIATA* ON CANINE DIROFILARIASIS

S. Ghosh, P. Sarkar, S. P. Sinha Babu and N. C. Sukul
Department of Zoology, Visva-Bharati University, Santiniketan-731 235, W.B.

A mixture (1:1) of the ethanol extracts from the leaves of *Centella asiatica* and the rhizomes of *Sansevieria trifasciata* was administered orally to pariah dogs naturally infected with *Dirofilaria immitis* at 36mg/kg/day for 45 days. The microfilarial density showed a 40.5% reduction following 30 days of treatment and a 51.7% reduction following 45 days of treatment. Discontinuation of treatment resulted in a rise of microfilarial level. There was no appreciable variation in mf density in the infected and untreated dogs.

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ANTIFILARIAL ACTIVITY OF *ARTEMISIA NILAGIRICA* AGAINST CANINE DIROFILARIASIS

P. Sarkar, S. P. Sinha Babu and N. C. Sukul
Department of Zoology, Visva-Bharati University, Santiniketan-731235, W.B.

Flowering meristems of *Artemisia nilagirica* collected from Shillong, were extracted with 90% ethanol at room temperature for one week. The solvent was removed by evaporation and the residue was dehydrated in a dessicator over anhydrous calcium chloride. Measured quantity of the residue were kept in gelatin capsules. Blood was sampled from 4 pariah dogs naturally infected with *Dirofilaria immitis* for two months. Microfilarial concentration was recorded in every sample. The dogs were then treated through oral route with the *Artemisia* extract at 10mg/kg/day for 15 days. Blood was sampled immediately after the first phase of treatment. After an interval of 15 days, the same dogs were treated again at a dose of 20mg/kg/day for 15 days. Blood was sampled again after the second phase of treatment. There was an average of 43.9% reduction in microfilarial concentration after the first phase of treatment. Following the second phase of treatment there was 78.6% reduction in mf concentration. The treated dogs did not show any symptoms of toxicity in the form of anorexia and change in their natural activity. It may be possible to eliminate the microfilaria in blood with increasing the dosage of the plant extract and the duration of treatment.

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IMMUNIZATION OF CATTLE AGAINST TICKS

D.P. Banerjee
Department of Parasitology, College of Veterinary Sciences, CCS, Haryana Agricultural University, Hisar 125 004, Haryana

Crossbred (*Bos taurus* x *Bos indicus*) cattles were immunized by repeated infestation, salivary gland antigens (SG Ags), whole tick extract antigen (WTE Ag) and passive transfer of plasma (obtained from SG Ag immunized calves). Challenge infestation of immunized calves was done by releasing 50 pairs (male and female) of *Hyalomma anatolicum anatolicum* ticks on ears. In repeated attachment experiment, significant differences were observed in parameters related to feeding and reproductive performance. Calves immunized with SG Ag-I and SG Ag-II after emulsification with Freund's complete adjuvant (FCA) showed significant acquired resistance. Significant decrease in engorged weight, egg mass weight and increase in preoviposition period was found in ticks on calves immunized with WTE Ag. Immunized sera recipient animals showed no significant resistance upon challenge with ticks. Capillary tube agglutination and double diffusion test gave positive reactions 21 days after first immunization in calves immunized with SG Ags. Per cent 'E' rosette were found increased significantly in repeatedly infested as well as SG Ags immunized calves. Histopathological studies at the tick attachment sites revealed formation of feeding cavity, cellular infiltration and epidermal vesicle. Intradermal inoculation of *H.marginatum isaaci* tick antigen showed immediate to delayed skin reactions in calves, infested repeatedly and immunized with SG Ags derived from partly fed *H.a. anatolicum* female adult ticks.

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IgG TITRE OF DOGS DURING THE PREPATENT PERIOD OF *ECHINOCOCCUS GRANULOSUS* INFECTION

Gul Ahmad and W.A. Nizami
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202 002

The F1 fraction of the protoscoleces of *E.granulosus* purified by Sephadex G-200 and immunoaffinity chromatography used antigen in the alkaline phosphatase micro-ELISA. The IgG level was monitored during the prepatent infection of dogs with *E. granulosus*. Specific antibody could be detected 4 days post infection in all the five animals experimentally infected with *E. granulosus*. The establishment of the infection was confirmed at necropsy 42 days post infection. It was found that a predictable rise in the IgG response could be detected in the sequentially collected sera of the experimentally infected dog puppies. A clear discriminating point between infected and uninfected (control) dogs have been established in the

present study. An ELISA value higher than 0.425 O.D. was the indication of the establishment of infection of *E. granulosus*. A very high trite of IgG could be detected when the sera diluted 1:1280 times. There was not much difference in the IgG titre among the five experimentally infected dogs used in the present study. The analyses of results indicate that IgG titre can be detected in the infected dogs very early during the prepatent period of infection. Therefore, ELISA may be used for immunodiagonosis of *E.granulosus* infection in dogs using F1 fraction of protoscoleces as antigen.

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PRELIMINARY STUDIES ON THE OCCURRENCE OF GELATINASES IN SOME DIGENETIC TREMATODES

Kavitha Singh, Wajih A. Nizami and S.M.A. Abidi
Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202 002

Gelatinases constitute an important group of proteases in helminths which might be involved in various physiological, biochemical and metabolic processes besides their role in tissue penetration. In a preliminary attempt, gelatinases in cell free homogenates of seven digenetic trematode species viz.: *Gastrothylax crumenifer, Fischoederius elongatus and Paramphistomum epiclitum* from rumen; *Explanatum explanatum* and *Fasciola gigantica* from liver of *Bubalus bubalis; Paramphistomum epiclitum* from rumen of goat; and *Isoporarchis hypselobagri* from the swim bladder of *Wallago attu* have been analysed by substrate gel zymography using 7-15% gradient SDS-polyacrylamide gels impregnated with 0.1% gelatin.

The zymograms of endogenous gelatinases revealed considerable differences in enzyme profile of the trematodes under study. The intergeneric and as well as interspecific variations in the number and intensity of substrate lytic zones was evident. The molecular weight (Mr) of majority of bands was detected in the range of 70-200 kDa. Maximum number of bands were detected in liverfluke *F.gigantica* while more pronounced gelatinolytic activity could be seen in *P. epiclitum* from goat. Strong enzyme activity was also evident in fish trematode *I. hypselobagri*. Whether such differences are consequence of occupying physiologically different microenvironment or due to the phyletic diversity reflecting the molecular heterogeneity in worms is difficult to conclude at this stage. Further studies are in progress to characterize the nature of these enzymes.

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EFFICACY OF ALBEN PLUS SUSPENSION 5% (ALBENDAZOLE 50 mg) AGAINST HAEMONCHOSIS IN SHEEP

R.D. Agarwal and P.P.S. Chauhan
Department of parasitology, College of Veterinary Science and Animal Husbandry, C.S. Azad, University of Agriculture and Technology, Campus, Mathura 281 001, U.P.

Faecal samples of two sheep, aged two years each belonging to the Department of Microbiology of the college were received for parasitological diagnosis. The ailing sheep showed marked clinical signs of anaemia, debility, diarrhoea with mucous and occasional streaks of blood. Faecal samples were examined by direct and centrifugation technique using Sheather's sugar solution and both were found positive for bursate worm infection. Eggs Per Gram (EPG) of faeces were estimated as 3200 and 4600, respectively . Next day, one sheep died, which on post-mortem examination exhibited large number of *Haemonchus contortus* from the abomasum. To the surviving sheep, Alben Plus Suspension 5% was given @ 15 mg/kg body weight, was administered orally. After treatment the faecal examination was done daily. It was observed that sheep became negative for *H.contortus* infection on 3rd day post treatment and EPG became zero. Thus the drug Alben Plus Suspension 5% (Jeps Pharma Pvt. Ltd., New Delhi was effective in clinical Haemonchosis condition in sheep.

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FIELD TRIAL ON THE EFFICACY OF BAYTICOL AGAINST IXODID TICKS IN CATTLE AND BUFFALOES

Ravindra Kumar, P.P.S. Chauhan, R.D. Agrawal and Daya Shankar
Department of Parasitology, College of Veterinary Science and Animal Husbandry, C.S. Azad, University of Agriculture and Technology, Mathura-Campus, Mathura 281 001 U.P.

Efficacy of Bayticol (Flumethrin 1%) pour-on preparation was tested against ixodid ticks (*Boophilus microplus, Hyalomma anatolicum anatolicum, H. marginatum isaaci* and *H. dromedarii*) in cattle and buffaloes, in a field trial. A total of 54 animals naturally infested with ixodid ticks were selected for the trial. These were divided into two groups, treated and untreated control with 27 animals (17 buffalos and 10 cows) in each. The animals in the treated group were treated with Bayticol pour-on solution (Flumethrin 1%) @ 1.0 mg/kg body weight. Measured quantity of the preparation was taken in a syringe and poured along the vertebral column from head to tail. The animals in the control group were applied with simple water by the same method. The count of ticks on each animal was recorded on day 0 (day of treatment) 1,2,5,10,20 and 30 day post-treatment. In the treated group of animals, no live tick could be detected on day-1 i.e. 24 hours after application of this drug. The dead

ticks that were left attached to the body of the treated animals looked flat, wrinkled, dried up and discoloured. The treated animals remained free of ticks till 30 day post-treatment. On the other hand, the untreated control group of animals continue to carry tick infestation till the end of the trial. Thus, Bayticol (Flumethrin 1%) pour-on preparation at the dose rate of 1 mg\kg body weight proved 100% effective against *Hyalomma* spp. and *Boophilus* sp. in cattle and buffaloes. The drug was quite safe, as no untoward effect was detected in any of treated animals.

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EFFECT OF DIFFERENT TEMPERATURES AND RELATIVE HIMIDITIES ON THE HATCHING OF THE EGGS OF TWO PIGEON LICE (PHTHIRAPTERA, INSECTA)

S.K. Singh, Adesh Kumar, Surman and A.K. Saxena
Department of Zoology. Government Postgraduate College, Kotdwara 246 149 Garhwal, U.P.

Eggs of two pigeon lice (Amblycera, *Colpocephalum turbinatum* and Ischnocera, *Columbicola columbae*) were reared at different temperatures and humidities, to record the physical environmental limits and to determine optimum conditions for hatching and incubation. Eggs of both the species normally hatch within 32 to 38°C. Higher temperatures reduces the viability as well as incubation period. Lower temperatures tend to prolong the incubation period. Relative humidities do not seem to have profound effect on hatching percentage and incubation period.

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ACARICIDAL EFFICACY OF AV/EPL/16 AGAINST SARCOPTIC MANGE IN BUFFALO CALVES

K.L. Khurana, S. Khanna, B. Kumar and R.K. Sethi
Central Institute for Research on Buffaloes, Sirsa Road, Hisar 125 001, Haryana

A herbal liquid formulation AV/EPL/16 was evaluated for acaricidal efficacy in natural cases of severe sarcoptic mange in buffalo calves. A single application of the recommended 1:4 dilution resulted in clinical improvement in all the twelve treated calves. Full clinical and parasitological cure was recorded in eleven calves 18 - 25 days post-treatment. Only one calf required a second treatment. Acaricidal effect was observed against all stages of the mites and residual protection lasted for three months. No adverse effect was seen inspite of licking from skin coat.

tion in resistance provides a better understanding of the mechanisms of the resistance. Comparison of responses in susceptible and resistant stock provides a powerful tool to distinguish among protective, irrelevant and pathological responses.

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A NEW RECORD OF *MONIEZIA (BLANCHARIEZIA) KALAWATI SP. NOV.* FROM *CAPRA HIRCUSL*

Sanjay Nanware*, Baba Jadhav and Mohan Babare***

** Post Graduate Department of Zoology, Arts, Science & Commerce College, Naldurg 413 602 Tq, Tuljapur, Dist. Osmannabad, M.S.*

*** University Department of Zoology, Dr. B.A. Marathwada University, Aurangabad 431 004, M.S.*

During routine postmortem examination, a domestic goat, *Capra hircus L.* Showed an unusual occurrence of *Moniezia (Blanchariezia) Kalawati Sp. Nov.* The morphological data of *Moniezia (Blanchariezia) Kalawati Sp. Nov.* infecting bovine intestine, have been dealt in detail. On morphological examination, scolex medium, almost squarish in shape with four suckers without rostellum, distinctly marked off from strobila, suckers small, oval, arranged in two pairs, one pair in each half region of scolex, lineally one overlapping the other in each pair at anterior most extremity of scolex. Neck short, more wide, matuare proglottids large, broader than long, almost five & half times broader than long, craspedote, each with double set of reproductive organs, craspedote with short, blunt projections at posterior corners of segments with convex irregular lateral margins. Testes small, oval, 172 in number, cirrus pouch medium, oval, broad in middle, narrow on both sides, obliquely placed, cirrus thin tube, with two to three curves, contained within cirrus pouch, vas deferens thin, short tube, coiled, long, runs with many short blunt acini placed in middle of segment, curved medially. Vagina thin tube, posterior to cirrus pouch, starts from genital pore, runs obliquely takes slight posterior turn reaches and opens into ootype. Ootype small, oval, situated antero-lateral to ovary. Vitelline gland small, compact lobe, postovarian just touching acini. Genital pores bilateral, small, oval. Longitudinal excretory canals are of medium width. Interproglottidal glands present; in inter segmental region of anterior and posterior margins of segments, medium, oval, 54 in number, highly muscular, either single or paired, irregularly arranged in central width of segments, leaving spaces on each lateral side, arranged lineally.

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COMPARATIVE STUDY OF BACTERIAL ISOLATES FROM DIFFERENT SEGMENTS OF GASTROINTESTINAL TRACT OF RODENTS

S.C. Nigam and H.B. Rajani

National Laboratory Animal Centre, Central Drug Research Institute, Lucknow 226 001

Routine microbiological monitoring of laboratory animals is of prime importance to keep the animal colony healthy. Using healthy animals for biomedical research will not only reduce the number required for the experiments but also generate an authentic and reproducible data. With this in view a comparative study of the prevalence of bacterial flora prevailing in different segments of gastrointestinal tract of various strains of rodent was undertaken. The study was conducted on 95 mice (Parks, Swiss, SMA, C3H / Jax, SJL / Jax, BALB / c, AKR), 108 rats (Druckrey, Sprague Dawley, Charels Foster, Fisher-344, Lew, Wistar), 16 mastomys, 15 cotton rats, 40 hamsters and 15 gerbils maintained at National Laboratory Animal centre (NLAC) of CDRI, Lucknow. The contents taken under aseptic condition from intestinal walls of various segments of G.I.T. were inoculated on MacConkey lactose Agar medium and plates incubated at 37^0C for 24-48 hrs. Isolates were confirmed on the basis of their cultural, morphological and biochemical characteristics. Various isolates viz., *Escherichia coli, Aerobacter spp., Klebsiella spp., Proteus mirabilis, Streptococcus faecalis, Staphylococcus spp.* and *Pseudomonas aeruginosa* from stomach, duodenum, jejunum, ileum and caecal parts of G.I.T. in various strains of rodents were identified. The highest percentage of *E. coli* isolated from stomach, duodenum, jejunum, ileum and caecum were, Druckrey rat (40), C3H / Jax mice (60), Swiss mice (50), Charles foster rats (80) and in BALB / c mice as well as in C.F. rats (85 each) respectively. Maximum percentage of *Aerobacter spp.* were in SMA mice (30) in stomach, duodenum and jejunum, whereas in ileum and caecum, it was in SMA mice (50) and BALB/c (50) respectively. Maximum percentage of *Klebsiella spp., Proteus mirabilis, Streptococcus faecalis and Staphylococcus spp.* in the stomach, duodenum, jejunum, ileum, and caecal parts were, in stomach as Druckrey rat (35), SMA mice (40) Charles foster rat (40), SMA mice (40) in duodenum C.F. rat (35), SMA mice (40), Charles foster (40), SMA mice (40), in jejunum - Swiss mice (40), Parkes mice (40), S.D. rats (40), BALB/c mice (33), in ileum as Lew rat (33), SMA mice (50), C.F. rat (50), S.D. rat (40) and in caecum, - BALB/c (60), hamster (50), hamster (50) and in Druckrey as well as Charles foster rats (30 each) respectively. *Pseudomonas aeruginosa* showed higher percentage in C3H/Jax mice in stomach, duodenum, jejunum, ileum and caecum as 20,20,20,30,30, respectively. The percentage of isolates were lower in mastomys, cotton rats and gerbil. The above study reveals segmental variations of isolates in different parts of gastrointestinal tract among the strains of rodents.

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PURIFICATION AND CHARACTERIZATION OF DNA TOPOISOMERASE II FROM FILARIAL PARASITE

J.K. Saxena, U. Pandya and O.P. Shukla
Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001

DNA topoisomerases are required for DNA replication, transcription and recombination. These enzymes have been utilized as primary cellular targets for some of the most widely prescribed antibiotics and anticancer drugs. The filarial parasite *Setaria cervi* contains DNA topoisomerase II. The enzyme was purified utilizing gel filtration and affinity chromatography. The purified enzyme showed the molecular weight of 75 kDa and it also utilized CTP, GTP, and UTP for enzyme activity. Topoisomerase inhibitors viz., nalidixic acid, ellipticine, novobiocin inhibited the enzyme activity to different extent. The antifilarial compounds, Diethylcarbamazine (DEC), Suramin and Ivermectin had significant inhibitory effect on the enzyme activity. The differences in kinetic properties of filarial enzyme can be utilized for design of effective antifilarial compounds for chemotherapy.

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SYNTHESIS OF BIS QUINOLINES AS POTENTIAL ANTIMALARIAL AGENTS

Swati Tewari[1], P.M.S. Chauhan[1], A.P. Bhaduri[1], Shalini Srivastava[2] and Subhash Chandra[2]
Division of Medicinal Chemistry[1] and Division of Parasitology[2], Central Drug Research Institute, Lucknow 226 001

Emergence of malaria parasites resistant to one or more classes of antimalarials has created problems to eradicate malaria by chemotherapy, Chloroquine resistance poses the most serious clinical problem especially with *P. falciparum*, which accounts for 85% of the cases and much of the mortality. Bisquinolines have recently been discovered to be the patent agent against chloroquine resistant malaria. It is also reported that being bulky in structure, they are less efficiently extraded by chloroquine resistant parasites as compared to chloroquine. Verampil and other "resistance modulator" drugs which reverse CQ resistance are believed to act by a blockade or inhibition of a putative drug transporter membrane protein (P-glycoprotein). This protein normally allows the CQ resistant parasites to pump CQ out of the cell. The effect of these "resistance modulators" on multidrug transporter proteins found in normal cells remains to be elucidated, however toxicity is noted with many of these drugs when used in combination with CQ. This toxicity may prove to be a significant obstacle in their therapeutic application. An alternative strategy which addresses the problem of CQ

resistance is drug design based on chemical entities known to be active against CQ resistant malaria. Thus, bisquinolines have been discovered as a new pharmacophore for combating the problem of chloroquine resistant malaria. Details of biological activity of bisquinolines will be discussed.

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INDUCTION OF GAMETOCYTOGENESIS IN CULTURED *PLASMODIUM CYNOMOLGI* BY ANTI - BLOOD STAGE MONOCLONAL ANTIBODIES

K.K. Kamboj
Division of Parasitology, Central Drug Research Institute, Lucknow 226 001

Plasmodium cynomolgi is regarded as a simian counterpart of the human malaria *P vivax*. Eleven different strains of *P. cynomolgi* have been isolated from different geographical regions of the world. Of these, the Berok strain has been found to be closest to *P. vivax* and is the only strain of *P. cynomolgi* which can be continuously cultured *in vitro*. It does not produce gametocytes under normal culture conditions. Monoclonal antibodies produced against the blood stages of this parasite were assessed for their effect on invasion of merozoite and intraerythrocytic development of the parasite. None of the monoclonal antibodies had any inhibitory effect on merozoite invasion. Some of the hybridomas were found to induct gametocytogenesis leading to the production of both male and female gametocytes.

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PREVALENCE AND LEVELS OF ANTIBODIES TO THE CIRCUMSPOROZOITE PROTEINS OF HUMAN MALARIA PARASITE

K.K. Kamboj, Roopali Chaturvedi, Vas Dev* and V.P. Sharma+
Division of Parasitology, Central Drug Research Institute, Lucknow 226 001
** Malaria Research Centre (FS), Sonapur 782 4002*
+ Malaria Research Centre, Delhi 110 054

Individuals living in malaria-endemic areas, exposed to *Plasmodium* sporozoites, develop antibodies directed primarily against the repetitive epitope of the CS protein. Synthetic peptides representing the repeats of the CS protein of the human malaria parasites were used to determine the anti-sporozoite antibody titres in Sonapur, Assam. Antisporozoite antibodies against *P. falciparum* and *P. vivax* were found at relatively high levels. In addition to the classical *P. vivax* strain, antibodies against the CS protein of VK 247 variant *P. vivax* were also found. Sera containing antibodies against the CS protein of *P. malariae* were found at a very low frequency.

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ANTIMALARIAL PROFILE OF AZITHROMYCIN AGAINST BLOOD AND SPOROZOITE INDUCED INFECTIONS IN MICE AND MONKEYS

S.K. Puri and Naresh Singh

Division of Microbiology, Central Drug Research Institute, Lucknow 226 001

The effective management of drug resistance in *P. falciparum* malaria which is now extending to *P. vivax* also is a steadily increasing clinical problem. Apart from efforts aimed to develop new drugs directed against novel parasite targets, the strategy to use other therapeutic agents to potentiate the effect of available antimalarial drugs can be useful in preventing the spread of drug resistant strains.

The spectrum of antimalarial activity of the new macrolide antibiotic azithromycin was evaluated against blood and sporozoite induced infections with a chloroquine resistant strain of *P. yoelii nigeriensis* (N-67) in Swiss mice and a simian parasite *P. cynomolgi* B in rhesus monkeys. Against experimental rodent malaria 70 mg/kg dose showed curative blood schizontocidal activity in the four dose regimen administered orally from day 0-3 or from day 2-5 to mice harbouring established infection. The curative response was also obtained with 40 mg/kg dose administered in extended 7 dose (day 0-6) regimen. Azithromycin was also effective in the causal prophylactic test, since 50 mg/kg dose from day -1 to +2 protected mice against *P. yoelii nigeriensis* (N-67) sporozoite challenge. In comparison, erythromycin did not show either of the above activities upto 405 mg/kg dose in identical regimens. Comparison of the ED_{90} values showed that azithromycin was 31 fold more effective than erythromycin as a blood schizontocide. In the simian model, trophozoite induced infections of *P. cynomolgi* B were cured with 25 mg/kg azithromycin administered for 7 days while prepatent period was significantly extended in monkeys challenged with *P. cynomolgi* B sporozoites presumably because of the inhibitory effect on primary exo-erythrocytic cycle. Azithromycin did not exhibit any hypnozoitocidal (dormant exo-erythrocytic stages) effect at 25 mg/kg x 7 day regimen.

The study shows usefulness of azithromycin as a prophylactic and therapeutic agent either alone or in combination with antimalarial drugs.

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STUDIES ON HOST SPECIFICITY OF MALARIA PARASITES OF RODENTS

Kavita Purbey and S.K. Puri
Division of Microbiology, Central Drug Research Institute, Lucknow 226 001

The host specificity of human malaria parasites represents a major constraint on the study of malaria as unlike the other major tropical diseases, the actual causative organism can not be maintained in small laboratory animals. The parasites of rodents have received a vast amount of attention both in their own right and as models for human malaria. The advantages of using rodent malarias include the easy maintainence of parasites. However, familiarity with the pattern of rodent infection in a wide variety of laboratory hosts is important since every species has its own particular characteristics and infection produced varies from host to host. We compared the infectivity, course of infection and virulence of four rodent parasites viz., *P. berghei* (NK-65), *P. yoelii nigeriensis* (N-67), *P. chabaudi chabaudi* and *P. vinckei* in four common laboratory rodents, namely, mice, rats, mastomys and hamsters, and results have shown wide variation in susceptibility of different hosts. *P. berghei* produced infection in all the four hosts with varied degree of mortality rate. *P. yoelii* was also found to produce sufficiently high parasitaemia levels in all the four hosts, although the infection was fatal only in mice. *P. vinckei* produced fulminating high infection in mice, transient low level infection in rats and mastomys and was not infective to hamsters. Infections with *P. chabaudi* produced most diverse pattern; in mice, 40-50% parasitaemia level was reached before declining on its own in majority of animals; a very low level transient infection developed in rats, and the parasites were not infective to mastomys and hamsters. Although observations on rodent malarias have provided useful clues to the nature of human disease, studies are envisaged to determine which rodent host provides the best model.

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CONCENTRATION AND TIME DEPENDENCY OF MEFLOQUINE EFFICACY AGAINST *PLASMODIUM FALCIPARUM IN VITRO*

S.D. Srinivas and S.K. Puri
Division of Microbiology, Central Drug Research Institute, Lucknow 226 001

The ability to characterize and quantify the pharmacodynamic measures of antimalarials *in vitro* affords a unique opportunity to link these observations with patient specific pharmacokinetic parameters to develop an optimal dosing regimen. Though such studies have been widely employed with antibiotics, it is still in its infancy as far as antimalarials are concerned presumably because of the delayed success in achieving continuous cultivation of malaria

parasites *in vitro*. We studied the concentration and time dependency of mefloquine efficacy against *P.falciparum* (FCD-3) *in vitro*. The parasites were exposed to three mefloquine concentrations viz. 200 ng/ml (1 MIC), 1000 ng/ml (5 MIC) and 5000 ng/ml (25 MIC) with exposure time ranging between 1-48 hours. The viability was assessed by cultivating drug exposed parasites in drug free medium for a limited duration of 144 hours post mefloquine exposure.

A complete inhibitory effect was observed when parasites were exposed at 200 ng/ml for 24 hours or more while exposure for upto 12 hours did not result in total loss of viability as growth continued in drug free medium. Parasites exposed to higher concentrations of 1000 ng/ml or 5000 ng/ml completely lost viability even after 3 hours of drug exposure.

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PRODUCTION OF ANTI-IDIOTYPIC ANTIBODIES TO PLASMODIUM SPECIFIC ANTI-LDH MONOCLONAL ANTIBODY

Neetu Tyagi, Nuzhat A. Kaushal and Deep C. Kaushal
Central Drug Research Institute, Lucknow 226 001

Malaria is a major public health problem affecting millions of people in tropical and sub-tropical countries. Considerable efforts have been directed towards control, prophylaxis and eventual eradication of malaria, however, the effective control of malaria will ultimately depend on rapid and accurate diagnosis of the disease. In view of the problems associated with parasitological diagnosis, emphasis has been given to immunological methods and antigen detection appears to be a better approach for diagnosing malaria. In our laboratory, we have developed a sensitive and specific immunodiagnostic test, based on detection of parasite lactate dehydrogenase (LDH), employing anti-LDH monoclonal antibodies. In the present study, the monoclonal antibody (Moab) against the plasmodial LDH was used to generate the anti-idiotypic antibodies in rabbits. The anti-plasmodial LDH monoclonal was affinity purified from the ascites fluid using protein-A sepharose affinity column. The rabbits were immunized with affinity purified anti-plasmodial LDH Moab. The affinity purified Moab was conjugated to peroxidase and used in inhibition ELISA for testing the immune rabbit sera. These studies revealed that immune rabbit sera inhibited specifically the binding of anti-LDH Moab to parasite LDH, thereby indicating the presence of anti-idiotypic antibodies in immune rabbit sera.

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PRESENCE OF HOST SERUM ALBUMIN IN EXCRETORY-SECRETORY PRODUCTS OF *SETARIA CERVI*

Huma Mustafa, Deep C. Kaushal and Nuzhat A. Kaushal
Central Drug Research Institute, Lucknow 226 001

Filariasis is a public health problem of major importance in tropical and subtropical countries. The immunological methods employing crude somatic or whole worm extracts yield false positive reactions due to the complexity of these antigens and their cross-reactivity with other helminth parasites. Excretory-secretory (E-S) antigens, released by the living parasites in the host, have been shown to be the better source of diagnostic antigens in comparison to somatic antigens. The characterization of E-S products is important not only for identifying the diagnostically important antigens but also to identify the proteins of host origin. In the present study, efforts were made to identify and remove the host serum contaminants from the E-S products of *Setaria cervi* (a bovine filarial parasite). The E-S products were obtained by maintaining the *S.cervi* adult worms in protein-free defined medium at 37^0 C for 32 h. Crossed immunoelecrophoretic (CIE) analysis, using rabbit anti-normal buffalo serum, revealed the presence of 3 precipitin peaks in *S.cervi* E-S products. Two of these precipitin peaks were identified as albumin bands using BSA and anti-BSA antibodies in crossed line immunoelectrophoresis. The host serum protein were removed by absorbing the *S.cervi* E-S products with anti-BSA sepharose.

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CHARACTERIZATION OF ANTI-IDIOTYPIC ANTIBODIES AGAINST A FILARIAL MONOCLONAL ANTIBODY

Ranjana Tripathi, Deep C. Kaushal and Nuzhat A. Kaushal
Central Drug Research Institute, Lucknow 226 001

The inhibition of idiotype-anti idiotype interactions have been used in parasitic diseases for diagnostic purposes. We have a monoclonal antibody (AB1) recognizing the filarial circulating antigen and, in order to develop a diagnostic test, efforts have been made to produce anti-idiotypic antibody against this monoclonal antibody (AB1). The anti-idiotypic antibodies (AB2) were generated in rabbits against the monoclonal antibody (AB1). The monoclonal antibody was purified from the ascites fluid and used for the immunization. The rabbits were given intra-muscular injection of AB1 emulsified in Freund's complete adjuvant at 15 days interval. The sera obtained from the immunized rabbits were tested in inhibition - ELISA and the immune rabbit sera were found to inhibit strongly the binding of peroxidase conjugated monoclonal antibody (AB1) to the filarial antigen. These findings suggest the presence of

anti-idiotypic antibody in immune rabbit serum. The immune rabbit sera will be used to purify the anti-idiotypic antibodies.

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REVERSAL OF IMMUNOCOMPROMISED/IMMUNOSUPPRESSED STATE WITH IMMUNOSTIMULANTS

Anju Puri, L.M.Tripathi, W.Haq and V.M.L. Srivastava
Central Drug Research Institute, Lucknow 226 001

Impairment or depression of immune system is known to be a common feature of several disorders ranging from AIDS to infectious diseases. The clinical need for agents to combat such conditions have led to the development of substances with immunostimulatory activity. Two structural analogues of muramyl dipeptide viz. 86/448 and 89/729 at this Institute have also been identified as potential stimulants after a series of *in vitro* and *in vivo* experiments. With an aim to examine their activity in immunosuppressed conditions, *in vitro* experiments were carried out with hydrocortisone (HC), a known immunosuppressor and active agent for stress. HC at 10^{-7} and 10^{-8} M concentrations caused nearly 50% inhibition of ConA induced proliferation of mouse lymphocytes. 86/448 and 89/729 as well as levamisole (taken as a standard) reversed the situation and nullified the effect of HC in a concentration dependent manner. 86/448 was further applied to *Leishmania* infected hamsters. It was interesting to note that the compound expressed both prophylactic and therapeutic effect in combination with stibogluconate. Application of these compounds against other infections is under investigation.

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EFFECT OF NUTRITIONAL SUPPLEMENTS AND PROTEASE INHIBITOR ON THE YIELD OF ES ANTIGENS OF *BRUGIA MALAYI* MICROFILARIAE *IN VITRO*

Bonny Bhunia, Rajshekhar Alli, M.V.R. Reddy and B.C. Harinath
Jamnalal Bajaj, Tropical Disease Research Centre and Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha 442 102

Brugia malayi microfilarial excretory-secretory (mf ES) antigens obtained by *in vitro* maintenance of mf are very useful in the immunodiagnosis of Bancroftian filariasis. The effect of additional supplements to the culture medium and the temperature were explored on the yield of ES products *in vitro*. Supplementation of RPMI-1640 medium with organic acids and sugars of Grace's insect culture medium for *in vitro* maintenance of 5 lakhs of mf in 40 ml medium increased the yield of mf ES protein content from 152 ug to 364 ug and the

antigen titre from 200 to 400. Supplementation with phenyl methyl sulphonyl fluoride (PMSF), a protease inhibitor and shift in the culture temperature from 37 to 28^{0}C resulted in further increase of mf ES antigen to 502 ug of total protein with an antigen titre of 800. Thus the modifications resulted in the net increase of 3 fold in the mean protein content and 4 fold in the mean antigen titre of ES products. The above modifications in the *in-vitro* maintenance of mf did not effect the diagnostic quality of mf ES antigen preparation which gave a sensitivity and specificify of 80 and 90% respectively in the detection of filarial IgG antibodies in *Wuchereria bancrofti* infected cases.

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DEVELOPMENT OF *IN VITRO* SCREENING SYSTEM FOR ANTIFILARIAL ASSAY

S. Rajkumar, S. Misra Bhattacharya and R.K. Chatterjee
Division of Parasitology, Central Drug Research Institute, Post Box 173, Lucknow 226 001

Thirty three plant products were tested *in vitro* at different concentrations viz. 250 µg/ml and 125 µg/ml against adult worms of *Acanthocheilonema viteae*, a rodent filariid. The parameters used for measuring antifilarial efficacy of plant products were the adverse effect on motility of the parasites and on the ability of exposed worms to metabolically reduce yellow coloured MTT [3-(4,5 dimethyl thiazol - 2 yl)-2,5 diphenyl tetrazolium bromide] to its product formazan, which can be estimated spectrophotometrically. The plant products which decreased the motility of worms to 1+ or made them completely immotile or caused more than 50% inhibition in reduction of MTT by the exposed parasites were considered as *in vitro* active. Among the five different concentrations 250 and 125 µg/ml were found to be very high concentrations resulting into death of worms in almost all extracts and 15.125 µg/ml appeared to be too low concentration where parasites remained unaffected. On the other hand, 62.5 and 31.25 µg/ml appeared as optimal concentrations. Amongst these two, 62.5 µg/ml has been picked up for routine screening to avoid missing any candidate plant product. The correlation between *in vitro* and *in vivo* antifilarial activity would also be discussed.

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CENTRAL GREY CATECHOLAMINERGIC INVOLVEMENT ON PAIN RESPONSIVENESS DURING FOOD DEPRIVATION IN CONSCIOUS RATS

Snehasis Bhunia
Department of Physiology, MGIMS, Sevagram 442 102

To determine the central grey catecholaminergic involvement on pain responsiveness, experiments were performed in conscious food deprived rats aged 90 days weighing 250-300 gms. They were restricted from food for 48 hrs. Pain threshold (phasic and emotional component of pain), heart rate (HR), respiratory rate (RR), were recorded during 12 hr. (post absorptive state), 24, 36 and 48 hr of food deprivation. Central microinfusion of noradrenaline phenoxybenzamine were given in central grey area during 12, 24, 36 and 48 hr of food deprivation and their effect on pain threshold, HR, RR were recorded. Pain threshold HR, RR were not altered during the early periods (12, 24, 36 hr) but the pain threshold was increased significantly during longer period of food deprivation. These results indicate that the pain responsiveness is uneffected during shorter period by central grey catecholaminergic involvement but the same is decreased significantly during the longer period of food deprivation.

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BEYOND BIO-TECHNOLOGY: THE EMERGING CHALLENGE OF SOCIA-EPIDEMIOLOGY*

Ravi Narayan
Society for Community Health Awareness, Research and Action, No. 367, Jakkasandra I Main, I Block, Koramangala, Bangalore 560 034

Research in vector control has focussed primarily on means to reduce vectors by chemical means and now increasingly on biological and environmental means. New 'biocides' and renewed interest in environmental manipulation methods have surfaced as the new significant developments. Vaccines are being developed. Biotechnology is the catch word and recent developments in this field have raised the promise of new exciting developments.

This short paper will raise however a new focus of research which the author feels is equally significant in its challenge - as is the area of Bio-technology - and that is socio-epidemiology. If the challenges of vector control have to make a significant impact at the community level and if the potential of bio-technology has to be fully realised than the biggest lacunae in the 'Lab to Land' transfer of new technology has to be urgently addressed - and that is inadequate and continuing neglect of the studies of human behaviour and the man-vector relationship.

The paper will highlight some of the lesser known studies and some of the interactive qualitative methods which need to be introduced into the agenda of research in the country so that this area of the problem is adequately focussed upon. It will draw on some case studies that were included in an expert group report process of which the author was Chairperson (Towards an Appropriate Malaria Control Strategy, VHAI/SOCHARA report, 1997).

A judicious balance between bio-technological research and socio-epidemiological research will increase our chances to win the battle against vector borne diseases. For this to happen - vector control researchers have to increasingly recognise the challenges of interdisciplinary problem analysis and work more closely with epidemiologists and behavioural scientists. The paper provides some suggestions for this.

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STUDIES IN THE RESISTANCE OF MALARIAL PARASITE TO ANTIMALARIAL DRUGS : A CASE STUDY OF NANDED CITY

P. Devendra Lingojwar
School of Life Sciences, S.R.T.M. University, Nanded 431 606

Resistance of *Plasmodium falciparum* (PF) to chloroquine is spreading rapidly in Eastern Africa and South Asia. It is reported that in India genetically 17 different strains are spreading due to migration. This increase in malaria has been the appearance of malarial parasite resistant to antimalarials like chloroquine and quinine. These drugs accumulate in the food vacuole of the parasite, where the proteolysis of ingested red cell hemoglobin takes place. The growing parasite obtain essential amino acids from the proteolysis of hemoglobin. Heme, a lysis product of hemoglobin interacts with biological membranes and inhibits different enzymes. Though heme is toxic to enzymatic system of parasite, by virtue of specific mechanism its detoxification takes place by forming a crystalline insoluble pigment called hemozoin.

The present study aims to estimate the amount of hemozoin before and after treatment with antimalarial e.g. chloroquine by a modified method. The estimated amount of hemozoin by our modified method is an indicator of resistance of *Plasmodium falciparum* towards antimalarials.

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MEDICINAL ASPECTS OF SOAP CONTAINING ESSENTIAL OILS (CAN A PARASITE CONTROL VECTOR & PARASITES?)

P.K. Shanbhag, S. Mallikarjuna and P.S. Anand
Karnataka Soaps and Detergents Ltd., Bangalore 560 055

Sandalwood tree native to India belongs to the family "Santalaceae". The fragrant sandalwood from the plant species *Santalumalbum.* Lin. is used for the extraction of sandalwood oil. Sandalwood and its oil are well known for their therapeutic values since ancient time. The golden yellow oil and its wood are being used for external as well as internal medicines for various diseases. Traditionally, sandalwood and its products including oil and soaps are known to control vectors by repulsion and or by killing. However, there seems to be no systematic research done on effective use of sandalwood and its products in control of vectors and parasites. In our considered view, being botanical parasite (Hemi parasite) by itself, systematic research would prove, this good drug to control vectors and parasites.

Similarly, other essential oils like lemon oil, geranium oil, eucalyptus oil, clove oil, pine oil, camphor, lavender oil etc, are useful medicinally which are made available to the consumer in the form of Soaps, Cosmetics, Creams, Perfumes etc.

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TRIBAL AND FOREST MALARIA IN ORISSA, INDIA

N. Mohapatra[1], R.K. Das[1], D.P. Hansdah[1], B.P. Patnaik[2], R.K. Hazra[1] and S.K. Parida[1]

1. Division of Medical Entomology and Parasitology, Regional Medical Research Centre (ICMR), Chandrasekharpur, Bhubaneswar 751 016

2. Regional Director, (Health & Family Welfare), BJ-25, B.J.B. Nagar, Bhubaneswar 751 014

Resurgence and increasing incidence of death due to malaria has been creating an alarming situation. Under varied geo-ecological conditions the occurrence, distribution and behavioural patterns of malaria vectors and the parasite are known to differ from place to place. The State Orissa is a part of peninsular India with varied geo-ecological conditions which are conducive for transmission of perennial malaria. Complexity and magnitude of malaria in Orissa deserves special attention as the State contributes 15 to 20% of total malaria in India. About 50% deaths due to malaria and more than 30% of falciparum malaria in India are reported from Orissa.

Malaria spreading among tribals has attracted special attention in India. About 22% of the population of Orissa is tribal. Tribals live in forests, foothills, hilltops and also in plains. There are tribal pockets all over Orissa, mostly in the undivided Districts of Koraput, Kalahandi, Mayurbhanj, Sundergarh, Ganjam and Phulbani. Among all the above Districts Koraput District has the maximum concentration of tribal population which constitute about 55.22% of total population of the District. About 75% of total malaria and 80% of the falciparum malaria in the State are reported from tribal areas.

This study aims at revealing the relationship between incidence of malaria in tribal and non-tribal PHCs' of Orissa, with special reference to *P. falciparum malaria*. A total of 314 PHCs' (out of which 157 Tribal PHCs') of 30 new Districts of Orissa State were taken as the samples of this study. District wise epidemiological data on malaria prevalence (from the year 1992 to 1996) were collected from health departments of the Government. The data are analyzed and presented.

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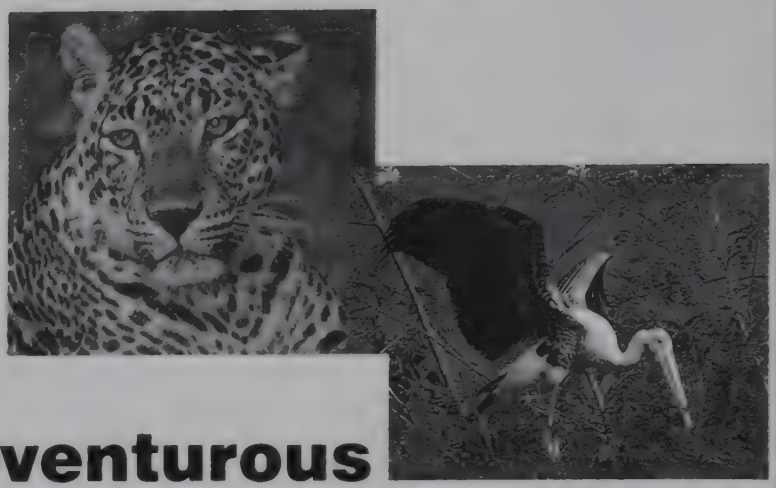

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ಏಡ್ಸ್ ಬಗ್ಗೆ ನೀವು ತಿಳಿದಿರಬೇಕಾದ ಮಾಹಿತಿ

ಏಡ್ಸ್ ಎಂದರೇನು?

ಏಡ್ಸ್ ಮನುಷ್ಯನ ಶಕ್ತಿಗುಂದಿಕೆಯ ಆರ್ಜಿತಲಕ್ಷಣ ಕೂಟ.

ಇದು ಹೆಚ್.ಐ.ವಿ. ರೋಗಾಣುವಿನ ಸೋಂಕಿನಿಂದ ಬರುವ ರೋಗ. ಹೆಚ್.ಐ.ವಿ. (ಹ್ಯೂಮನ್ ಇಮ್ಯುನೋ ಡಿಫಿಷಿಯೆನ್ಸಿ ವೈರಸ್) ದೇಹದಲ್ಲಿರುವ ರೋಗ ನಿರೋಧಕ ಶಕ್ತಿಯನ್ನು ನಾಶಮಾಡುತ್ತದೆ, ನಂತರ ಆ ಮನುಷ್ಯನು ಬೇರೆ ಬೇರೆ ರೋಗಗಳಿಗೆ ಬೇಗನೆ ತುತ್ತಾಗುತ್ತಾನೆ.

ಏಡ್ಸ್ ರೋಗ ಹರಡುವ ರೀತಿ :

- ☐ ಏಡ್ಸ್ ರೋಗದ ಸೋಂಕಿರುವ ಪುರುಷ ಅಥವಾ ಸ್ತ್ರೀಯರೊಂದಿಗೆ ಲೈಂಗಿಕ ಸಂಪರ್ಕ ಹೊಂದುವುದರಿಂದ ಹರಡುತ್ತದೆ.
- ☐ ಬಹುವ್ಯಕ್ತಿಗಳೊಂದಿಗಿನ ಲೈಂಗಿಕ ಸಂಪರ್ಕ ಹೊಂದಿದರೆ ಈ ರೋಗ ಹರಡುವ ಸಾಧ್ಯತೆಗಳು ಹೆಚ್ಚು.
- ☐ ಸೋಂಕಿರುವ ವ್ಯಕ್ತಿಯ ರಕ್ತವನ್ನು ಪರೀಕ್ಷಿಸದೆ ಬೇರೆಯವರಿಗೆ ಕೊಟ್ಟಾಗ ಈ ರೋಗ ಬರುತ್ತದೆ.
- ☐ ರೋಗದ ಸೋಂಕಿರುವ ವ್ಯಕ್ತಿಗೆ ಉಪಯೋಗಿಸಿದ ಸೂಜಿ, ಸಿರಿಂಜ್‌ಗಳು ಸರಿಯಾಗಿ ಶುದ್ಧೀಕರಿಸದೇ ಸಂಸ್ಕರಿಸದೇ ಇತರರಿಗೆ ಬಳಸುವುದರಿಂದ ಹರಡುತ್ತದೆ.
- ☐ ಸೋಂಕುಳ್ಳ ತಾಯಿಯಿಂದ ತನ್ನ ಹುಟ್ಟಲಿರುವ ಮಗುವಿಗೆ ಈ ಸೋಂಕು ಹರಡುತ್ತದೆ.

ಏಡ್ಸ್ ರೋಗದ ಲಕ್ಷಣಗಳು :

- ☐ ಏಡ್ಸ್ ಸೋಂಕು ತಗುಲಿದ 3 ತಿಂಗಳಿಂದ 15 ವರ್ಷದ ಅವಧಿಯಲ್ಲಿ ಏಡ್ಸ್ ರೋಗ ಲಕ್ಷಣ ಕಾಣಿಸಿಕೊಳ್ಳುತ್ತದೆ. ಏಡ್ಸ್ ರೋಗ ಲಕ್ಷಣಗಳು ಕಾಣಿಸಿಕೊಳ್ಳುವವರೆಗೆ ಎಲ್ಲರಂತೆ ಆರೋಗ್ಯವಾಗಿ ಕಾಣುತ್ತಾರೆ. ಆದರೆ ಈ ಅವಧಿಯಲ್ಲಿ ಇವರು ಬೇರೆಯವರೊಂದಿಗೆ ಮೇಲಿನ ವಿಧಾನಗಳಲ್ಲಿ ಸೋಂಕನ್ನು ಹರಡುವ ಶಕ್ತಿಯುಳ್ಳವರಾಗಿರುತ್ತಾರೆ.
- ☐ ಯಾವುದೇ ಕಾರಣವಿಲ್ಲದೆ ಶರೀರದ ತೂಕ ಶೇ. 10ಕ್ಕಿಂತಲೂ ಕಡಿಮೆಯಾಗುವುದು.
- ☐ 1 ತಿಂಗಳಿಗಿಂತಲೂ ಹೆಚ್ಚಾಗಿ ಕಂಡುಬರುವ ಅತಿಸಾರ (ಭೇದಿ)
- ☐ 1 ತಿಂಗಳಿಗಿಂತಲೂ ಹೆಚ್ಚಾಗಿ ಕಂಡುಬರುವ ಕೆಮ್ಮು
- ☐ 1 ತಿಂಗಳಿಗಿಂತಲೂ ಹೆಚ್ಚು ಕಾಲದಿಂದಿರುವ ಜ್ವರ ಬಾಧೆ
- ☐ ಕತ್ತು ಕಂಕುಳು ಮತ್ತಿತರ ಜಾಗದಲ್ಲಿ ದುಗ್ಧ-ರಸ ಗ್ರಂಥಿಗಳ (ಗಡ್ಡೆ) ಊತ
- ☐ ಬಾಯಿ ಮತ್ತು ಗಂಟಲನಲ್ಲಿ ವಾಸಿಯಾಗದ ಹುಣ್ಣು
- ☐ ಸಾಮಾನ್ಯವಾಗಿ ಕಂಡುಬರುವ ಚರ್ಮ ವ್ಯಾಧಿಗಳು

ತಡೆಗಟ್ಟುವ ಬಗೆ :

- ☐ ಪರಸ್ಪರ ವಿಶ್ವಾಸಾರ್ಹರಾಗಿರುವ ಒಬ್ಬರೊಂದಿಗೆ ಮಾತ್ರ ಜೀವನ ಪರ್ಯಂತ ಲೈಂಗಿಕ ಸಂಬಂಧ ಹೊಂದಿರುವುದು
- ☐ ಅಪರಿಚಿತ ಸ್ತ್ರೀ ಅಥವಾ ಪುರುಷರೊಂದಿಗೆ ಲೈಂಗಿಕ ಸಂಪರ್ಕ ಇಟ್ಟುಕೊಳ್ಳಬಾರದು
- ☐ ರಕ್ತದಾನ ಪಡೆಯುವ ಸಂದರ್ಭಗಳಲ್ಲಿ ಹೆಚ್.ಐ.ವಿ. ಸೋಂಕು ರಹಿತವೆಂದು ಪರೀಕ್ಷೆಯಿಂದ ಖಚಿತಪಡಿಸಿದ ರಕ್ತವನ್ನೇ ಉಪಯೋಗಿಸುವುದು
- ☐ ಒಮ್ಮೆ ಒಬ್ಬರಿಗೆ ಉಪಯೋಗಿಸಿದ ಸೂಜಿ, ಸಿರೆಂಜ್ ಇತ್ಯಾದಿ ಉಪಕರಣಗಳನ್ನು ಸಂಸ್ಕರಿಸಿ ಶುದ್ಧೀಕರಿಸಿ ನಂತರ ಬಳಸುವುದು
- ☐ ಏಡ್ಸ್ ಮತ್ತು ಲೈಂಗಿಕ ರೋಗಗಳನ್ನು ತಡೆಯಲು ಲೈಂಗಿಕ ಕ್ರಿಯೆಯಲ್ಲಿ ನಿರೋಧ್ ಉಪಯೋಗಿಸುವುದರಿಂದ ತಡೆಗಟ್ಟಬಹುದು

ಏಡ್ಸ್ ರೋಗಿಗಳ ಬಗ್ಗೆ ಅನಾದರ ಬೇಡ :

- ☐ ಏಡ್ಸ್ ರೋಗವು ಸಾಮಾನ್ಯ ದಿನನಿತ್ಯದ ಸಂಪರ್ಕಗಳಾದ ಕೈಕುಲುಕುವುದು, ಜೊತೆಯಲ್ಲಿ ವಾಸ ಮಾಡುವುದು, ತಬ್ಬಿಕೊಳ್ಳುವುದು, ಕೂಡಿ ಊಟಮಾಡುವುದು, ಟವಲ್ ಉಪಯೋಗಿಸುವುದು ಇತ್ಯಾದಿಗಳ ಮೂಲಕ ಹರಡದೇ ಇರುವುದರಿಂದ ಅವರನ್ನು ಪ್ರೀತಿ, ವಿಶ್ವಾಸದಿಂದ ಎಲ್ಲಾ ರೋಗಿಗಳಂತೆ ಕಾಣುವುದು
- ☐ ಏಡ್ಸ್ ರೋಗಿಗೆ ಶಾರೀರಿಕ ಆರೈಕೆ ಮತ್ತು ಮಾನಸಿಕ ಆಸರೆ ಇವೆರಡರ ಅಗತ್ಯವಿದೆ
- ☐ ಏಡ್ಸ್ ರೋಗಿಗಳು ಶಾಂತಿ, ಸಮಾಧಾನದಿಂದ ಜೀವಿಸಲು ಅವಕಾಶ ಕಲ್ಪಿಸಿಕೊಡುವುದು ಮಾನವೀಯತೆಯ ಧರ್ಮ

ಏಡ್ಸ್ ರೋಗ ತಡೆಗಟ್ಟಲು ಈವರೆವಿಗೂ ಯಾವುದೇ ಲಸಿಕೆ ಲಭ್ಯವಿಲ್ಲ ಹಾಗೂ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ನಿರ್ದಿಷ್ಟವಾದ ಔಷಧಿ ಇಲ್ಲ. ಏಡ್ಸ್ ಬಾರದಂತೆ ಮುಂಜಾಗ್ರತಾ ಕ್ರಮ ಅನುಸರಿಸುವುದು ಒಂದೇ ಮಾರ್ಗ

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